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 * * * * * * * * * *     Welcome to STN International     * * * * * * * * * *

 NEWS  1              Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2              "Ask CAS" for self-help around the clock
 NEWS  3   SEP 09     CA/CAplus records now contain indexing from 1907 to the
                      present
 NEWS  4   DEC 08     INPADOC: Legal Status data reloaded
 NEWS  5   SEP 29     DISSABS now available on STN
 NEWS  6   OCT 10     PCTFULL: Two new display fields added
 NEWS  7   OCT 21     BIOSIS file reloaded and enhanced
 NEWS  8   OCT 28     BIOSIS file segment of TOXCENTER reloaded and enhanced
 NEWS  9   NOV 24     MSDS-CCOHS file reloaded
 NEWS 10   DEC 08     CABA reloaded with left truncation
 NEWS 11   DEC 08     IMS file names changed
 NEWS 12   DEC 09     Experimental property data collected by CAS now available
                      in REGISTRY
 NEWS 13   DEC 09     STN Entry Date available for display in REGISTRY and CA/CAplus
 NEWS 14   DEC 17     DGENE: Two new display fields added
 NEWS 15   DEC 18     BIOTECHNO no longer updated
 NEWS 16   DEC 19     CROPU no longer updated; subscriber discount no longer
                      available
 NEWS 17   DEC 22     Additional INPI reactions and pre-1907 documents added to CAS
                      databases
 NEWS 18   DEC 22     IFIPAT/IFIUDB/IFICDB reloaded with new data and search fields
 NEWS 19   DEC 22     ABI-INFORM now available on STN
 NEWS 20   JAN 27     Source of Registration (SR) information in REGISTRY updated
                      and searchable
 NEWS 21   JAN 27     A new search aid, the Company Name Thesaurus, available in
                      CA/CAplus
 NEWS 22   FEB 05     German (DE) application and patent publication number format
                      changes
 NEWS 23   MAR 03     MEDLINE and LMEDLINE reloaded
 NEWS 24   MAR 03     MEDLINE file segment of TOXCENTER reloaded
 NEWS 25   MAR 03     FRANCEPAT now available on STN

 NEWS EXPRESS   MARCH 5 CURRENT WINDOWS VERSION IS V7.00A, CURRENT
                MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP),
                AND CURRENT DISCOVER FILE IS DATED 3 MARCH 2004
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 NEWS WWW       CAS World Wide Web Site (general information)

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FILE 'HOME' ENTERED AT 18:59:44 ON 10 MAR 2004

=> file uspatful
COST IN U.S. DOLLARS                               SINCE FILE      TOTAL
                                                        ENTRY    SESSION
FULL ESTIMATED COST                                      0.21       0.21

FILE 'USPATFULL' ENTERED AT 19:00:06 ON 10 MAR 2004
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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 9 Mar 2004 (20040309/PD)
FILE LAST UPDATED: 9 Mar 2004 (20040309/ED)
HIGHEST GRANTED PATENT NUMBER: US6704933
HIGHEST APPLICATION PUBLICATION NUMBER: US2004045070
CA INDEXING IS CURRENT THROUGH 9 Mar 2004 (20040309/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 9 Mar 2004 (20040309/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Dec 2003
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Dec 2003

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>>>  published document but also a list of any subsequent              <<<
>>>  publications.  The publication number, patent kind code, and      <<<
>>>  publication date for all the US publications for an invention     <<<
>>>  are displayed in the PI (Patent Information) field of USPATFULL   <<<
>>>  records and may be searched in standard search fields, e.g., /PN, <<<
>>>  /PK, etc.                                                         <<<

>>>  USPATFULL and USPAT2 can be accessed and searched together        <<<
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>>>  classifications, or claims, that may potentially change from      <<<
>>>  the earliest to the latest publication.                           <<<

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=> e yang yeasing y/in
E1           2      YANG YEA YUN/IN
E2          11      YANG YEASING/IN
E3           4 --> YANG YEASING Y/IN
E4           1      YANG YEE/IN
E5           1      YANG YEH HO/IN
E6           1      YANG YEN LIN/IN
E7           2      YANG YEN SHENG/IN
E8           3      YANG YEN SHUO/IN
E9           1      YANG YENNI/IN
E10          1      YANG YEON DAE/IN
E11          1      YANG YEON S/IN
E12          1      YANG YEONG F/IN

=> s e3
L1           4 "YANG YEASING Y"/IN

=> d l1,ti,1-4

L1    ANSWER 1 OF 4  USPATFULL on STN
TI      Amplification of HIV-1 sequences for detection of sequences associated
        with drug-resistance mutations

L1    ANSWER 2 OF 4  USPATFULL on STN
TI      Detection of HIV-1 by nucleic acid amplification

L1    ANSWER 3 OF 4  USPATFULL on STN
TI      Compositions and methods for detecting human immunodeficiency virus 2
        (HIV-2)

L1    ANSWER 4 OF 4  USPATFULL on STN
TI      Amplification of HIV-1 sequences for detection of sequences associated
```

=> d l1,cbib,ab,clm,1-4

L1 ANSWER 1 OF 4 USPATFULL on STN
2003:324599 Amplification of HIV-1 sequences for detection of sequences associated with drug-resistance mutations.
Yang, Yeasing Y., San Diego, CA, UNITED STATES
Brentano, Steven T., Santee, CA, UNITED STATES
Babola, Odile, Decines, FRANCE
Tran, Nathalie, Dagneux, FRANCE
Vernet, Guy, Albigny sur Saone, FRANCE
US 2003228574 A1 20031211
APPLICATION: US 2003-425975 A1 20030428 (10)
PRIORITY: US 2000-229790P 20000901 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Sequences of nucleic acid oligonucleotides for amplifying different portions of gag and pol genes of HIV-1 and for detecting such amplified nucleic acid sequences are disclosed. Methods of amplifying and detecting HIV-1 nucleic acid in a biological sample using the amplification oligonucleotides specific for gag and pol target sequences are disclosed.
CLM What is claimed is:
1. A nucleic acid oligomer for amplifying a nucleotide sequence of HIV-1, comprising a sequence selected from the group consisting of SEQ ID NO:5 to SEQ ID NO:22 and SEQ ID NO:33 to SEQ ID NO:68.

2. A nucleic acid oligomer according to claim 1, wherein the oligomer nucleic acid backbone comprises one or more 2'-O-methoxy linkages, peptide nucleic acid linkages, phosphorothioate linkages, methylphosphonate linkages or any combination of these linkages.

3. A nucleic acid oligomer according to claim 1, wherein the oligomer is a promoter-primer comprising a sequence selected from the group consisting of SEQ ID NO:5 to SEQ ID NO:10 and SEQ ID NO:33 to SEQ ID NO:45, wherein a 5' portion of the sequence includes a promoter sequence for T7 RNA polymerase.

4. A mixture of nucleic acid oligomers according to claim 1, wherein the mixture comprises oligomers for amplifying a first gag sequence and having a nucleotide sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:11, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:17 and SEQ ID NO:59.

5. A mixture of nucleic acid oligomers according to claim 1, wherein the mixture comprises oligomers for amplifying a second gag sequence and having a nucleotide sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:18, and SEQ ID NO:60.

6. A mixture of nucleic acid oligomers according to claim 1, wherein the mixture comprises oligomers for amplifying a Protease sequence and having a nucleotide sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:19, SEQ ID NO:61, and SEQ ID NO:62.

7. A mixture of nucleic acid oligomers according to claim 1, wherein the mixture comprises oligomers for amplifying a first reverse transcriptase (RT) sequence and having a nucleotide sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:14, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:20, SEQ ID NO:63, SEQ ID NO:64 and SEQ ID NO:65.

8. A mixture of nucleic acid oligomers according to claim 1, wherein the

mixture comprises oligomers for amplifying a second RT sequence and having a nucleotide sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:15, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:21, and SEQ ID NO:66.

9. A mixture of nucleic acid oligomers according to claim 1, wherein the mixture comprises oligomers for amplifying a third RT sequence and having a nucleotide sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:16, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:22, SEQ ID NO:67, and SEQ ID NO:68.

10. A labeled oligonucleotide that specifically hybridizes to an HIV-1 sequence derived from gag or pol sequences, having a base sequence selected from the group consisting of SEQ ID NO:23 to SEQ ID NO:29, and a label that results in a detectable signal.

11. A labeled oligonucleotide according to claim 10, wherein the oligonucleotide includes in its nucleic acid backbone one or more 2'-O-methoxy linkages, peptide nucleic acid linkages, phosphorothioate linkages, methylphosphonate linkages or any combination these linkages.

12. A labeled oligonucleotide according to claim 10, wherein the label is a compound that produces a luminescent signal that can be detected in a homogeneous detection system.

13. A labeled oligonucleotide according to claim 10, wherein the label is an acridinium ester (AE) compound and the oligonucleotide hybridizes to an HIV-1 sequence derived from gag sequences and has a base sequence selected from the group consisting of: SEQ ID NO:23, SEQ ID NO:24 and SEQ ID NO:25.

14. A labeled oligonucleotide according to claim 10, wherein the label is an acridinium ester (AE) compound and the oligonucleotide hybridizes to an HIV-1 sequence derived from pol sequences and has a base sequence selected from the group consisting of: SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29.

15. A method of detecting HIV-1 in a biological sample, comprising the steps of: providing a biological sample containing HIV-1 nucleic acid; mixing the sample with two or more amplification oligomers that specifically amplify at least one HIV-1 target sequence contained within gag and pol sequences under conditions that allow amplification of nucleic acid, wherein the amplification oligomers have sequences selected from the group consisting of: SEQ ID NO:5, SEQ ID NO:11, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:17, and SEQ ID NO:59 to amplify a first gag sequence; SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:18, and SEQ ID NO:60 to amplify a second gag sequence; SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:19, SEQ ID NO:61 and SEQ ID NO:62 to amplify a first pol sequence, which is a protease sequence; SEQ ID NO:8, SEQ ID NO:14, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:20, SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65 to amplify a second pol sequence, which is a first reverse transcriptase (RT) sequence; SEQ ID NO:9, SEQ ID NO:15, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:21, and SEQ ID NO:66 to amplify a third pol sequence, which is a second RT sequence; and SEQ ID NO:10, SEQ ID NO:16, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:22, SEQ ID NO:67, and SEQ ID NO:68 to amplify a fourth pol sequence, which is a third RT sequence; or a combination of oligomers selected from these groups that allows amplification of at least one gag sequence and at least pol sequence; amplifying the target sequence to produce an amplified nucleic acid product; and detecting the presence of the amplified nucleic acid product.

16. The method of claim 15, wherein the amplifying step uses a

transcription mediated amplification method which is conducted in substantially isothermal conditions.

17. The method of claim 15, wherein the detecting step uses: a labeled oligomer having the sequence of SEQ ID NO:23, SEQ ID NO:24 or SEQ ID NO:25, or a mixture of these oligomers, to hybridize specifically to the amplified nucleic acid produced from a gag sequence; a labeled oligomer having the sequence of SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:29, or a mixture of these oligomers, to hybridize specifically to the amplified nucleic acid produced from a pol sequence; or a mixture of at least two labeled oligomers, wherein the mixture comprises one or more first labeled oligomers selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25, and one or more second labeled oligomers selected from the group consisting of SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29 to hybridize specifically to the amplified nucleic acid produced from at least one gag and at least one pol sequence.

18. The method of claim 15, wherein the detecting step detects hybridization of the amplified nucleic acid to an array of nucleic acid probes.

19. The method of claim 15, further comprising the step of contacting the sample containing HIV-1 nucleic acid with at least one capture oligomer having a sequence that hybridizes specifically to the HIV-1 nucleic acid, thus forming a hybridization complex that includes the HIV-1 nucleic acid and separating the hybridization complex from other sample components.

L1 ANSWER 2 OF 4 USPATFULL on STN
2003:253523 Detection of HIV-1 by nucleic acid amplification.
Bee, Gary G., Vista, CA, United States
Yang, Yeasing Y., San Diego, CA, United States
Kolk, Dan, Ramona, CA, United States
Giachetti, Cristina, Solana Beach, CA, United States
McDonough, Sherrol H., San Diego, CA, United States
Gen-Probe Incorporated, San Diego, CA, United States (U.S. corporation)
US 6623920 B1 20030923
APPLICATION: US 2000-611627 20000707 (9)
PRIORITY: US 1999-143072P 19990709 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Nucleic acid sequences and methods for detecting HIV-1 nucleic acid (LTR and pol sequences) in biological samples by detecting amplified nucleic acids are disclosed. Kits comprising nucleic acid oligomers for amplifying HIV-1 nucleic acid present in a biological sample and detecting the amplified nucleic acid are disclosed.
CLM What is claimed is:
1. A method of detecting HIV-1 nucleic acid in a biological sample, comprising the steps of: providing a biological sample containing HIV-1 nucleic acid; contacting the biological sample with at least one capture oligomer comprising a base sequence that hybridizes specifically to a target region in LTR or pol sequences of HIV-1 nucleic acid, thus forming a capture oligomer:HIV-1 nucleic acid complex; separating the capture oligomer:HIV-1 nucleic acid complex from the biological sample; then amplifying the LTR or pol sequences, or a cDNA made therefrom, using a nucleic acid polymerase in vitro to produce an amplified product; and detecting the amplified product using a labeled detection probe that hybridizes specifically with the amplified product, thereby indicating presence of the HIV-1 nucleic acid in the biological sample.

2. The method of claim 1, wherein the contacting step uses a capture oligomer further comprising a tail sequence that binds to a complementary sequence immobilized on a solid support.

3. The method of claim 1, wherein the base sequence of the capture oligomer that hybridizes specifically to a target region in the LTR sequence is a sequence of about 22 to about 54 bases including about 22 bases consisting essentially of LTR-specific bases contained in SEQ ID NO:2.

4. The method of claim 1, wherein the capture oligomer comprises an LTR-specific oligomer of about 22 to about 55 bases including about 22 bases consisting essentially of LTR-specific bases contained in SEQ ID NO:2 and optionally one or more oligomers that hybridize specifically to a target region in the pol sequence.

5. The method of claim 4, wherein the capture oligomer is a combination of at least two oligomers wherein one oligomer is an LTR-specific oligomer of about 22 to about 55 bases including about 22 bases consisting essentially of LTR-specific bases contained in SEQ ID NO:2 and at least one oligomer that hybridizes specifically to a pol sequence.

6. The method of claim 1, wherein the amplifying step uses at least two amplification oligomers that bind specifically to LTR or pol sequences or sequences complementary to LTR or pol sequences.

7. The method of claim 6, wherein the amplifying step uses amplification oligomers for amplifying LTR sequences consisting essentially of SEQ ID NO:7 and SEQ ID NO:9.

8. The method of claim 6, wherein the amplifying step uses at least two amplification oligomers for amplifying pol sequences consisting essentially of sequences of: SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:14.

9. The method of claim 1, wherein the amplifying step comprises a transcription-associated amplification method that uses: at least one promoter-primer comprising a promoter sequence that is recognized by an RNA polymerase when the promoter sequence is double stranded, wherein the promoter sequence is covalently attached to the 5' end of a LTR-specific sequence consisting essentially of SEQ ID NO:7, or a pol-specific sequence consisting essentially of SEQ ID NO:12 or SEQ ID NO:14; and at least one primer selected from a LTR-specific sequence consisting essentially of SEQ ID NO:9, or a pol-specific sequence consisting essentially of SEQ ID NO:10 or SEQ ID NO:11, provided that the LTR-specific promoter-primer is combined with the LTR-specific primer for amplifying a LTR target region, and/or at least one pol-specific promoter-primer is combined with at least one pol-specifics primer for amplifying a pol target region.

10. The method of claim 9, wherein the amplifying step uses any of the following combinations of promoter-primers and primers: promoter-primers consisting essentially of SEQ ID NO:12 and SEQ ID NO:14, with primers consisting essentially of SEQ ID NO:10 and SEQ ID NO:11; promoter-primers consisting essentially of SEQ ID NO:7, SEQ ID NO:12 and SEQ ID NO:14, with primers consisting essentially of SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:11; a promoter-primer consisting essentially of SEQ ID NO:12, and a primer consisting essentially of SEQ ID NO:10; or a promoter-primer consisting essentially of SEQ ID NO:7, and a primer consisting essentially of SEQ ID NO:9.

11. The method of claim 1, wherein the detecting step uses at least one labeled detection probe having a base sequence selected from: a LTR-specific sequence consisting essentially of SEQ ID NO:16, a pol-specific sequence consisting essentially of SEQ ID NO:17, or a pol-specific sequence consisting essentially of SEQ ID NO:18.

12. The method of claim 1, wherein the detecting step uses a combination of at least two labeled detection probes, wherein the probe base

sequences are about 20 bases consisting essentially of SEQ ID NO:16, about 22 to about 30 bases consisting essentially of SEQ ID NO:17, or about 17 to about 20 bases consisting essentially of SEQ ID NO:18.

13. The method of claim 12, wherein the labeled detection probe consisting essentially of SEQ ID NO:16 has an inosine at position 7.

14. The method of claim 1, wherein the detecting step uses a labeled detection probe of about 20 bases consisting essentially of SEQ ID NO:16.

15. The method of claim 1, wherein the detecting step uses at least one labeled detection probe of about 22 to about 30 bases consisting essentially of SEQ ID NO:17, or about 17 to about 20 bases consisting essentially of SEQ ID NO:18.

16. The method of claim 1, wherein the detecting step uses at least one labeled detection probe that includes at least one 2'-methoxy backbone linkage.

17. The method of claim 1, wherein: the contacting step uses capture oligomers made up of LTR-specific bases consisting essentially of about 22 LTR-specific bases contained in SEQ ID NO:2 and a tail sequence of about 5 to 50 bases that are not LTR-specific and provide a means of capturing HIV-1 nucleic acid hybridized to the LTR-specific sequence from the other components in the biological sample; the amplifying step uses promoter-primers consisting essentially of the sequences of SEQ ID NO:7, SEQ ID NO:12 and SEQ ID NO:14 and primers consisting essentially of the sequences of SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:11; and the detecting step uses labeled detection probes wherein probe base sequences are about 20 bases consisting essentially of SEQ ID NO:16, about 22 to about 30 bases consisting essentially of SEQ ID NO:17 and about 17 to about 20 bases consisting essentially of SEQ ID NO:18.

18. The method of claim 1, wherein: the contacting step uses at least two capture oligomers that hybridize to different sequences in the target region; the amplifying step uses at least two different promoter-primers that hybridize to a first set of sequences within the target region and at least two different primers that hybridize to a second set of sequences within the target region; and the detecting step uses at least two labeled probes that bind specifically to different sequences located between the first set and second set of sequences within the target region.

19. The method of claim 18, wherein: the contacting step uses capture oligomers having sequences that hybridize specifically to pol sequences; the amplifying step uses promoter-primers consisting essentially of sequences of SEQ ID NO:12 and SEQ ID NO:14 and primers consisting essentially of the sequences of SEQ ID NO:10 and SEQ ID NO:11; and the detecting step uses labeled probes of about 22 to about 30 bases consisting essentially of SEQ ID NO:17 and about 17 to about 20 bases consisting essentially of SEQ ID NO:18.

20. The method of claim 18, wherein the amplifying step uses at least two promoter-primers that hybridize to a first set of overlapping sequences within the target region, at least two primers that hybridize to a second set of overlapping sequences within the target region, or at least two promoter-primers that hybridize to a first set of overlapping sequences within the target region and at least two primers that hybridize to a second set of overlapping sequences within the target region.

21. A kit comprising a combination of oligomers, wherein the oligomers contained in the kit have sequences consisting essentially of SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:17 and SEQ ID NO:18, and wherein the oligomers consisting essentially of the sequences of SEQ ID

NO:17 and SEQ ID NO:18 are labeled with a detectable label.

22. The kit of claim 21, also containing oligomers with base sequences consisting essentially of the sequences of SEQ ID NO:7, SEQ ID NO:9 and SEQ ID NO:16, wherein the oligomer consisting essentially of SEQ ID NO:16 is labeled with a detectable label.

23. The method of claim 21, wherein the oligomer consisting essentially of SEQ ID NO:16 has an inosine at position 7.

24. A composition comprising a combination of at least two oligomers selected from the group consisting of: SEQ ID NO:7, optionally with a promoter sequence covalently attached to the 5' end of SEQ ID NO:7; and SEQ ID NO;9.

25. The composition of claim 24, wherein the promoter sequence is a T7 RNA polymerase promoter sequence.

26. The composition of claim 24, wherein SEQ ID NO:7 with the promoter sequence covalently attached to the 5' end is SEQ ID NO:8.

27. The composition of claim 24, wherein the composition further comprises an oligomer of SEQ ID NO:16 containing an inosine base.

28. The composition of claim 27, wherein an oligomer base sequence is linked by a backbone that includes at least one 2'-methoxy RNA group, at least one 2' fluoro-substituted RNA group, at least one peptide nucleic acid linkage, at least one phosphorohioate linkage, at least one methylphosphonate linkage or any combination thereof.

29. The composition of claim 27, wherein the oligomer of SEQ ID NO:16 containing an inosine base comprises at least one 2'-methoxy RNA group in the backbone.

30. The composition of claim 27, wherein the oligomer of SEQ ID NO:16 containing an inosine base comprises a detectable label joined directly or indirectly to the oligomer.

31. The composition of claim 30, wherein the detectable label is a chemiluminescent compound.

32. An oligomer consisting of SEQ ID NO:1, wherein the oligomer includes at least one 2'-methoxy RNA group, and wherein SEQ ID NO:1 may be optionally covalently joined with a 3' homopolymeric tail of about 30 bases.

33. A composition comprising a combination of at least two oligomers selected from the group consisting of: SEQ ID NO:12, optionally with a promoter sequence covalently attached to the 5' end of SEQ ID NO:12; SEQ ID NO:14, optionally with a promoter sequence covalently attached to the 5' end of SEQ ID NO:14; SEQ ID NO:10; and SEQ ID NO: 11.

34. The composition of claim 33, wherein the combination further comprises at least one oligomer selected from the group consisting of: SEQ ID NO:17, and SEQ ID NO:18 containing an inosine base.

35. The composition of claim 34, wherein an oligomer base sequence is linked by a backbone that includes at least one 2'-methoxy RNA group, at least one 2' fluro-substituted RNA group, at least one peptide nucleic acid linkage, at least one phosphorothioate linkage, at least one methylphosphonate linkage or any combination thereof.

36. The composition of claim 34, wherein the oligomer of SEQ ID NO:17 or SEQ ID NO:18 containing an Inosine base comprises at least one 2'-methoxy RNA group in the backbone.

37. The composition of claim 37, wherein the oligomer of SEQ ID NO:17 or SEQ ID NO:18 containing an inosine base comprises a detectable label joined directly or indirectly to the oligomer.

38. The composition of claim 37, wherein the detectable label is a chemiluminescent compound.

L1 ANSWER 3 OF 4 USPATFULL on STN
2002:314651 Compositions and methods for detecting human immunodeficiency virus 2 (HIV-2).
Yang, Yeasing Y., San Diego, CA, UNITED STATES
Burrell, Terrie A., San Diego, CA, UNITED STATES
US 2002177127 A1 20021128
APPLICATION: US 2001-1407 A1 20011022 (10)
PRIORITY: US 2000-242620P 20001023 (60)
US 2001-280058P 20010330 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compositions and methods for synthesizing and detecting HIV-2 specific amplicons. Particularly described are oligonucleotides that are useful as hybridization probes, and amplification primers that facilitate detection of very low levels of HIV-2 nucleic acids.

CLM What is claimed is:

1. A composition for detecting an HIV-2 nucleic acid sequence, comprising: a first amplification oligonucleotide comprising a sequence of 9-34 contiguous bases contained within the sequence of SEQ ID NO:9, said first amplification oligonucleotide having a length of up to 100 nucleotides; and a second amplification oligonucleotide comprising a sequence of 19-40 contiguous bases from the sequence of SEQ ID NO: 1, said second amplification oligonucleotide having a length of up to 100 nucleotides.

2. The composition of claim 1, wherein the length of the second amplification oligonucleotide is 19-40 nucleotides.

3. The composition of claim 2, wherein the length of the first amplification oligonucleotide is 18-60 nucleotides.

4. The composition of claim 3, wherein the length of the first amplification oligonucleotide is 18-34 nucleotides.

5. The composition of claim 4, wherein the length of the first amplification oligonucleotide is 18-25 nucleotides.

6. The composition of claim 5, wherein the sequence of the first amplification oligonucleotide is selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO:12, SEQ ID NO:13 and SEQ ID NO:14.

7. The composition of claim 2, wherein the length of the first amplification oligonucleotide is 18-60 nucleotides, and wherein the first amplification oligonucleotide further comprises a promoter sequence.

8. The composition of claim 2, wherein the length of the second amplification oligonucleotide is 19-21 nucleotides.

9. The composition of claim 8, wherein the length of the first amplification oligonucleotide is 18-34 nucleotides.

10. The composition of claim 3, wherein the length of the second amplification oligonucleotide is 19-21 nucleotides.

11. The composition of claim 7, wherein the first amplification oligonucleotide is a promoter-primer selected from the group consisting

NO:19.

12. The composition of claim 10, wherein the second amplification oligonucleotide is selected from the group consisting of SEQ'ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7.

13. The composition of claim 10, wherein the first amplification oligonucleotide further comprises a promoter sequence.

14. The composition of claim 13, wherein the first amplification oligonucleotide is a promoter-primer selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO:17, SEQ ID NO:18 and SEQ ID NO:19.

15. The composition of claim 13, wherein the second amplification oligonucleotide is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7.

16. The composition of claim 14, wherein the second amplification oligonucleotide is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7.

17. The composition of claim 1, wherein the length of the first amplification oligonucleotide is 18-25 nucleotides, and wherein the length of the second amplification oligonucleotide is 19-21 nucleotides.

18. The composition of claim 17, wherein the first amplification oligonucleotide is selected from the group consisting of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13 and SEQ ID NO:14.

19. The composition of claim 17, wherein the second amplification oligonucleotide is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7.

20. The composition of claim 18, wherein the second amplification oligonucleotide is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7.

21. The composition of claim 1, further comprising an oligonucleotide detection probe having a sequence that comprises SEQ ID NO:21 or the complement thereof.

22. The composition of claim 21, wherein said oligonucleotide detection probe has a length of up to 18 nucleotides.

23. The composition of claim 22, wherein the sequence of said oligonucleotide detection probe is selected from the group consisting of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26 and SEQ ID NO:27.

24. The composition of claim 23, wherein the sequence of the first amplification oligonucleotide is selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18 and SEQ ID NO:19, wherein the sequence of the second amplification oligonucleotide is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7, and wherein the sequence of the oligonucleotide detection probe is selected from the group consisting of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26 and SEQ ID NO:27.

25. A method for determining whether a biological sample comprising nucleic acids includes an HIV-2 nucleotide base sequence, said method comprising the steps of: contacting the nucleic acids of the biological sample with a composition comprising, a first amplification

within the sequence of SEQ ID NO:9, said first amplification oligonucleotide having a length of up to 100 nucleotides, and a second amplification oligonucleotide comprising a sequence of 19-40 contiguous bases from the sequence of SEQ ID NO:1, said second amplification oligonucleotide having a length of up to 100 nucleotides; amplifying any of said HIV-2 nucleotide base sequence present in said biological sample to produce amplified nucleic acids; and detecting said amplified nucleic acids produced in the amplifying step, whereby detection of said amplified nucleic acids indicates that said biological sample included the HIV-2 nucleotide base sequence.

26. The method of claim 25, wherein the length of the first amplification oligonucleotide is 18-60 nucleotides, and wherein the length of the second amplification oligonucleotide is 19-40 nucleotides.

27. The method of claim 26, wherein said first amplification oligonucleotide is a promoter-primer, and wherein the amplifying step comprises amplifying by TMA.

28. The method of claim 26, wherein the detecting step comprises first hybridizing the amplified nucleic acids with a hybridization assay probe specific for said amplified nucleic acids, and thereafter measuring an amount of said hybridization assay probe that hybridized said amplified nucleic acids.

29. The method of claim 28, wherein the hybridization assay probe is a labeled nucleic acid probe.

30. The method of claim 28, wherein the hybridization assay probe comprises the sequence of SEQ ID NO:21 or the complement thereof, said hybridization assay probe having a length of up to 22 nucleotides.

31. An oligonucleotide comprising the sequence of SEQ ID NO:21 or the complement thereof and a detectable label, said oligonucleotide having a length of up to 35 nucleotides.

32. The oligonucleotide of claim 31, wherein the length of said oligonucleotide is up to 22 nucleotides.

33. The oligonucleotide of claim 32, having at least 16 contiguous nucleotides contained within the sequence of SEQ ID NO:20 or the complement thereof.

34. The oligonucleotide of claim 33, wherein said oligonucleotide has the sequence of SEQ ID NO:20 or the complement thereof.

35. The oligonucleotide of claim 33, wherein said oligonucleotide has a length of up to 18 nucleotides.

36. The oligonucleotide of claim 35, wherein the length of said oligonucleotide is 18 nucleotides.

37. The oligonucleotide of claim 35, wherein said oligonucleotide has a sequence selected from the group consisting of SEQ ID NO:22 or the complement thereof, SEQ ID NO:23 or the complement thereof, SEQ ID NO:24 or the complement thereof, SEQ ID NO:25 or the complement thereof, SEQ ID NO:26 or the complement thereof, and SEQ ID NO:27 or the complement thereof.

38. The oligonucleotide of claim 31, wherein said oligonucleotide comprises DNA.

39. The oligonucleotide of claim 31, wherein said oligonucleotide comprises at least one nucleotide analog.

nucleotide analog comprises a methoxy group at the 2° position of a ribose moiety.

41. The oligonucleotide of claim 37, wherein the detectable label is a chemiluminescent label or a radiolabel.

42. The oligonucleotide of claim 41, wherein the detectable label is an acridinium ester.

43. A method for detecting the presence of HIV-2 nucleic acids in a biological sample, comprising the steps of: (a) providing to said biological sample a hybridization probe comprising the sequence of SEQ ID NO:21 or the complement thereof and a detectable label, said oligonucleotide having a length of up to 35 nucleotides; (b) hybridizing under a high stringency condition any HIV-2 nucleic acid that may be present in the biological sample with said hybridization probe to form a probe:target duplex; and (c) detecting said probe:target duplex as an indicator of the presence of HIV-2 in the biological sample.

44. The method of claim 43, wherein the length of the hybridization probe in the providing step is up to 22 nucleotides.

45. The method of claim 44, wherein said biological sample is a blood product selected from the group consisting of plasma and serum.

46. The method of claim 45, wherein before step (a) there is a step for releasing nucleic acid from any HIV-2 that may be present in said biological sample.

47. The method of claim 46, further comprising a step for capturing onto a solid support the nucleic acid released from said any HIV-2 that may be present in said biological sample.

48. The method of claim 44, wherein said biological sample is a lysate.

49. The method of claim 44, wherein said high stringency hybridization condition comprises 0.48 M sodium phosphate buffer, 0.1% sodium dodecyl sulfate, and 1 mM each of EDTA and EGTA.

50. The method of claim 44, wherein said high stringency hybridization condition comprises a salt concentration in the range of 0.6 - 0.9 M.

51. The method of claim 44, wherein the hybridization probe in step (a) has a sequence selected from the group consisting of SEQ ID NO:22 or the complement thereof, SEQ ID NO:23 or the complement thereof, SEQ ID NO:24 or the complement thereof, SEQ ID NO:25 or the complement thereof, SEQ ID NO:26 or the complement thereof, and SEQ ID NO:27 or the complement thereof.

52. The method of claim 51, wherein the hybridization probe comprises at least one nucleotide analog.

53. The method of claim 51, wherein the hybridization probe comprises a detectable label.

54. The method of claim 53, wherein the detectable label is an acridinium ester, and wherein the detecting step comprises performing luminometry to detect any of said probe:target duplex.

55. A kit for detecting HIV-2 nucleic acids, comprising: (a) a first amplification oligonucleotide comprising a sequence of 9-34 contiguous bases contained within the sequence of SEQ ID NO:9, said first amplification oligonucleotide having a length of up to 100 nucleotides; and (b) a second amplification oligonucleotide comprising a sequence of

amplification oligonucleotide having a length of up to 100 nucleotides.

56. The kit of claim 55, further comprising: (c) an oligonucleotide detection probe that comprises the sequence of SEQ ID NO:21 or the complement thereof, and a detectable label.

L1 ANSWER 4 OF 4 USPATFULL on STN

2002:105890 Amplification of HIV-1 sequences for detection of sequences associated with drug-resistance mutations.

Yang, Yeasing Y., San Diego, CA, UNITED STATES
Brentano, Steven T., Santee, CA, UNITED STATES
Babola, Odile, Decines, FRANCE
Tran, Nathalie, Dagneux, FRANCE
Vernet, Guy, Albigny sur Saone, FRANCE
US 2002055095 A1 20020509
APPLICATION: US 2001-944036 A1 20010831 (9)
PRIORITY: US 2000-229790P 20000901 (60)
DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Sequences of nucleic acid oligonucleotides for amplifying different portions of gag and pol genes of HIV-1 and for detecting such amplified nucleic acid sequences are disclosed. Methods of amplifying and detecting HIV-1 nucleic acid in a biological sample using the amplification oligonucleotides specific for gag and pol target sequences are disclosed.

CLM What is claimed is:

1. A nucleic acid oligomer for amplifying a nucleotide sequence of HIV-1, comprising a sequence selected from the group consisting of SEQ ID NO:5 to SEQ ID NO:22 and SEQ ID NO:33 to SEQ ID NO:68.

2. A nucleic acid oligomer according to claim 1, wherein the oligomer nucleic acid backbone comprises one or more 2'-O-methoxy linkages, peptide nucleic acid linkages, phosphorothioate linkages, methylphosphonate linkages or any combination of these linkages.

3. A nucleic acid oligomer according to claim 1, wherein the oligomer is a promoter-primer comprising a sequence selected from the group consisting of SEQ ID NO:5 to SEQ ID NO:10 and SEQ ID NO:33 to SEQ ID NO:45, wherein a 5' portion of the sequence includes a promoter sequence for T7 RNA polymerase.

4. A mixture of nucleic acid oligomers according to claim 1, wherein the mixture comprises oligomers for amplifying a first gag sequence and having a nucleotide sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:11, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:17 and SEQ ID NO:59.

5. A mixture of nucleic acid oligomers according to claim 1, wherein the mixture comprises oligomers for amplifying a second gag sequence and having a nucleotide sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:18, and SEQ ID NO:60.

6. A mixture of nucleic acid oligomers according to claim 1, wherein the mixture comprises oligomers for amplifying a Protease sequence and having a nucleotide sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:19, SEQ ID NO:61, and SEQ ID NO:62.

7. A mixture of nucleic acid oligomers according to claim 1, wherein the mixture comprises oligomers for amplifying a first reverse transcriptase (RT) sequence and having a nucleotide sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:14, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:20, SEQ ID

NO:63, SEQ ID NO:64 and SEQ ID NO:65.

8. A mixture of nucleic acid oligomers according to claim 1, wherein the mixture comprises oligomers for amplifying a second RT sequence and having a nucleotide sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:15, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:21, and SEQ ID NO:66.

9. A mixture of nucleic acid oligomers according to claim 1, wherein the mixture comprises oligomers for amplifying a third RT sequence and having a nucleotide sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:16, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:22, SEQ ID NO:67, and SEQ ID NO:68.

10. A labeled oligonucleotide that specifically hybridizes to an HIV-1 sequence derived from gag or pol sequences, having a base sequence selected from the group consisting of SEQ ID NO:23 to SEQ ID NO:29, and a label that results in a detectable signal.

11. A labeled oligonucleotide according to claim 10, wherein the oligonucleotide includes in its nucleic acid backbone one or more 2'-O-methoxy linkages, peptide nucleic acid linkages, phosphorothioate linkages, methylphosphonate linkages or any combination these linkages.

12. A labeled oligonucleotide according to claim 10, wherein the label is a compound that produces a luminescent signal that can be detected in a homogeneous detection system.

13. A labeled oligonucleotide according to claim 10, wherein the label is an acridinium ester (AE) compound and the oligonucleotide hybridizes to an HIV-1 sequence derived from gag sequences and has a base sequence selected from the group consisting of: SEQ ID NO:23, SEQ ID NO:24 and SEQ ID NO:25.

14. A labeled oligonucleotide according to claim 10, wherein the label is an acridinium ester (AE) compound and the oligonucleotide hybridizes to an HIV-1 sequence derived from pol sequences and has a base sequence selected from the group consisting of: SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29.

15. A method of detecting HIV-1 in a biological sample, comprising the steps of: providing a biological sample containing HIV-1 nucleic acid; mixing the sample with two or more amplification oligomers that specifically amplify at least one HIV-1 target sequence contained within gag and pol sequences under conditions that allow amplification of nucleic acid, wherein the amplification oligomers have sequences selected from the group consisting of: SEQ ID NO:5, SEQ ID NO:11, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:17, and SEQ ID NO:59 to amplify a first gag sequence; SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:18, and SEQ ID NO:60 to amplify a second gag sequence; SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:19, SEQ ID NO:61 and SEQ ID NO:62 to amplify a first pol sequence, which is a protease sequence; SEQ ID NO:8, SEQ ID NO:14, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:20, SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65 to amplify a second pol sequence, which is a first reverse transcriptase (RT) sequence; SEQ ID NO:9, SEQ ID NO:15, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:21, and SEQ ID NO:66 to amplify a third pol sequence, which is a second RT sequence; and SEQ ID NO:10, SEQ ID NO:16, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:22, SEQ ID NO:67, and SEQ ID NO:68 to amplify a fourth pol sequence, which is a third RT sequence; or a combination of oligomers selected from these groups that allows amplification of at least one gag sequence and at least pol sequence; amplifying the target sequence to produce an amplified nucleic acid product; and detecting the presence

of the amplified nucleic acid product.

16. The method of claim 15, wherein the amplifying step uses a transcription-mediated amplification method which is conducted in substantially isothermal conditions.

17. The method of claim 15, wherein the detecting step uses: a labeled oligomer having the sequence of SEQ ID NO:23, SEQ ID NO:24 or SEQ ID NO:25, or a mixture of these oligomers, to hybridize specifically to the amplified nucleic acid produced from a gag sequence; a labeled oligomer having the sequence of SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:29, or a mixture of these oligomers, to hybridize specifically to the amplified nucleic acid produced from a pol sequence; or a mixture of at least two labeled oligomers, wherein the mixture comprises one or more first labeled oligomers selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25, and one or more second labeled oligomers selected from the group consisting of SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29 to hybridize specifically to the amplified nucleic acid produced from at least one gag and at least one pol sequence.

18. The method of claim 15, wherein the detecting step detects hybridization of the amplified nucleic acid to an array of nucleic acid probes.

19. The method of claim 15, further comprising the step of contacting the sample containing HIV-1 nucleic acid with at least one capture oligomer having a sequence that hybridizes specifically to the HIV-1 nucleic acid, thus forming a hybridization complex that includes the HIV-1 nucleic acid and separating the hybridization complex from other sample components.

```
=> e burrell terrie a/in
E1          12      BURRELL ROBERT E/IN
E2          20      BURRELL ROBERT EDWARD/IN
E3           1 --> BURRELL TERRIE A/IN
E4           1      BURRELL THEODORE ALEXANDER/IN
E5           2      BURRELL TIMOTHY/IN
E6           3      BURRELL TRAVIS/IN
E7           3      BURRELL WAYNE R/IN
E8           1      BURRELL WILLIAM A/IN
E9           3      BURRELL WILLIAM E/IN
E10          1      BURRELL WILLIAM ELDON/IN
E11          1      BURRELLO DANIEL M/IN
E12          1      BURRELLO DAVID T/IN

=> s e3 or e4
             1 "BURRELL TERRIE A"/IN
             1 "BURRELL THEODORE ALEXANDER"/IN
L2           2 "BURRELL TERRIE A"/IN OR "BURRELL THEODORE ALEXANDER"/IN

=> d l2,ti,1-2

L2    ANSWER 1 OF 2  USPATFULL on STN
TI      Compositions and methods for detecting human immunodeficiency virus 2
        (HIV-2)

L2    ANSWER 2 OF 2  USPATFULL on STN
TI      MANUFACTURE OF FRUIT BASKETS AND THE LIKE


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COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                           ENTRY    SESSION
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MOST RECENT DERWENT UPDATE:      200417          <200417/DW>
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    SDIS USING THE TIME RANGE CODE WILL NEED TO BE UPDATED.
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=> e yang yeasing y/in
E1           24     YANG Y Y/IN
E2            1     YANG Y Y C/IN
E3            0 --> YANG YEASING Y/IN
E4            2     YANG YU X/IN
E5         1153     YANG Z/IN
E6            1     YANG Z B/IN
E7            1     YANG Z G/IN
E8            1     YANG Z H/IN
E9            3     YANG Z J/IN
E10           3     YANG Z P/IN
E11           3     YANG Z Q/IN
E12           1     YANG Z S H J/IN

=> s e1
L3           24 "YANG Y Y"/IN

=> s l3 and (HIV-1 or HIV-2 or HIV)
         17191 HIV
       7229519 1
          2631 HIV-1
                 (HIV(W)1)
         17191 HIV
       4915364 2
           854 HIV-2
                 (HIV(W)2)
         17191 HIV
L4            5 L3 AND (HIV-1 OR HIV-2 OR HIV)

=> d l4,bib,ab,1-5

L4   ANSWER 1 OF 5  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN   2004-060998 [06]   WPIDS
CR   2002-462902 [49]
DNC  C2004-025022
TI   New nucleic acid oligomer for amplifying and detecting HIV-1
     nucleotide sequences and in providing information about the infective
```

agent, e.g. genetic subgroup or drug resistance phenotype based on
detectable sequence information.
DC B04 D16
IN BABOLA, O; BRENTANO, S T; TRAN, N; VERNET, G; **YANG, Y Y**
PA (BABO-I) BABOLA O; (BREN-I) BRENTANO S T; (TRAN-I) TRAN N; (VERN-I) VERNET G; (YANG-I) YANG Y Y
CYC 1
PI US 2003228574 A1 20031211 (200406)* 39p
ADT US 2003228574 A1 Provisional US 2000-229790P 20000901, Div ex US 2001-944036 20010831, US 2003-425975 20030428
FDT US 2003228574 A1 Div ex US 6582920
PRAI US 2000-229790P 20000901; US 2001-944036 20010831; US 2003-425975 20030428
AB US2003228574 A UPAB: 20040123
NOVELTY - A nucleic acid oligomer for amplifying a nucleotide sequence of **HIV-1**, is new. The nucleic acid oligomer comprises any of the 55 fully defined nucleotide sequences of 17-55 bp given in the specification.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a mixture of nucleic acid oligomers cited above;

(2) a labeled oligonucleotide that specifically hybridizes to an **HIV-1** sequence derived from gag or pol sequences, having a base sequence selected from any of the 7 fully defined nucleotide sequences of 18-25 bp given in the specification, and a label that results in a detectable signal; and

(3) a method of detecting **HIV-1** in a biological sample.

USE - The composition and method are useful in amplifying and detecting **HIV-1** nucleic acid sequences and in providing additional information about the infective agent, such as its genetic subgroup or drug-resistance phenotype based on detectable sequence information.
Dwg.0/2

L4 ANSWER 2 OF 5 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2002-489953 [52] WPIDS
DNC C2002-139091
TI Detecting human immunodeficiency virus-2 nucleic acids in a sample, by amplifying nucleic acids with oligonucleotides and detecting nucleic acid, or hybridizing nucleic acid with a probe and detecting probe target duplex.
DC B04 D16
IN BURRELL, T A; **YANG, Y Y**
PA (GENP-N) GEN-PROBE INC; (BURR-I) BURRELL T A; (YANG-I) YANG Y Y
CYC 97
PI WO 2002034951 A2 20020502 (200252)* EN 58p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PH PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2002030545 A 20020506 (200257)
US 2002177127 A1 20021128 (200281)
ADT WO 2002034951 A2 WO 2001-US45396 20011022; AU 2002030545 A AU 2002-30545 20011022; US 2002177127 A1 Provisional US 2000-242620P 20001023, Provisional US 2001-280058P 20010330, US 2001-1407 20011022
FDT AU 2002030545 A Based on WO 2002034951
PRAI US 2001-280058P 20010330; US 2000-242620P 20001023; US 2001-1407 20011022
AB WO 200234951 A UPAB: 20020815
NOVELTY - Detecting (M) human immunodeficiency virus-2 nucleic acids (NAs) in a biological sample, by contacting NAs with 1st and 2nd amplification oligonucleotides, amplifying **HIV-2** nucleotide base sequence, and detecting the amplified NA, or providing a hybridization probe and detectable label, hybridizing **HIV-2** NA with the probe to form probe:target duplex, and detecting the duplex, is new.

(**HIV-2**) nucleic acids (NAs) in a biological sample (BS) involves:

(a) contacting NAs of BS with a composition comprising, a first amplification oligonucleotide (ONT1) comprising 9-34 contiguous bases of the sequence S1, with a length of up to 100 nucleotides, and a second amplification oligonucleotide (ONT2) comprising 19-40 contiguous bases from the sequence S2, with a length of 100 nucleotides;

(b) amplifying any of the **HIV-2** nucleotide base sequences present in BS to produce amplified NAs; and

(c) detecting the amplified NAs which indicates that BS included the **HIV-2** nucleotide base sequence.

Alternatively, the method involves:

(a) providing to BS, a hybridization probe comprising the sequence (S3) GTCTGTTAGGACCC or its complement, with a length of 35 nucleotides, and a detectable label;

(b) hybridizing under a high stringency condition any **HIV-2** NA present in BS with the hybridization probe to form a probe:target duplex; and

(c) detecting the probe:target duplex as an indicator of the presence of **HIV-2** in BS.

INDEPENDENT CLAIMS are also included for the following:

(1) a composition (I) for detecting an **HIV-2** NA sequence, comprising ONT1 and ONT2;

(2) an oligonucleotide (II) comprising (S3) or its complement, with a length of 35 nucleotides, and a detectable label; and

(3) a kit for detecting **HIV-2** NAs, comprising ONT1 and ONT2.

GTCCCTGTTCGGGCGCCAACCTGCTAGGGATTTT (S1)

GTGTGTGTTCCCATCTCTCCTAGTCGCCGCCTGGTCATTC (S2)

USE - (M) is useful for detecting the presence of **HIV-2** NAs in a lysate or a blood product such as plasma or serum (claimed), and also for detecting subtypes A, B, C and D of **HIV-2**. (I) is useful for amplifying and detecting the NA in BS and also as components of multiplex amplification reactions that synthesize amplicons corresponding to polynucleotides of unrelated viruses, e.g. **HIV-1**, hepatitis B virus (HBV) and hepatitis C virus (HCV).

ADVANTAGE - (M) is a convenient and highly sensitive method.

Dwg.0/1

L4 ANSWER 3 OF 5 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2002-462902 [49] WPIDS
CR 2004-060998 [06]
DNC C2002-131439
TI New nucleic acid oligomers for amplifying a nucleotide sequence from **HIV-1** and probes for detecting the amplified product are specific for gag and pol regions and are useful to detect different subtypes of **HIV-1**.
DC B04 D16
IN BABOLA, O; BRENTANO, S T; TRAN, N; VERNET, G; **YANG, Y Y**
PA (BABO-I) BABOLA O; (BREN-I) BRENTANO S T; (TRAN-I) TRAN N; (VERN-I) VERNET G; (YANG-I) YANG Y Y; (INMR) BIOMERIEUX SA; (GENP-N) GEN-PROBE INC
CYC 1
PI US 2002055095 A1 20020509 (200249)* 19p
US 6582920 B2 20030624 (200343)
ADT US 2002055095 A1 Provisional US 2000-229790P 20000901, US 2001-944036 20010831; US 6582920 B2 Provisional US 2000-229790P 20000901, US 2001-944036 20010831
PRAI US 2000-229790P 20000901; US 2001-944036 20010831
AB US2002055095 A UPAB: 20040123

NOVELTY - Nucleic acid oligomers for amplifying a nucleotide sequence of **HIV-1** are new.

DETAILED DESCRIPTION - The nucleic acid oligomer for amplifying a nucleotide sequence from **HIV-1**, comprises a 50, 22, 54 or 22 nucleotide sequence fully defined in the specification.

INDEPENDENT CLAIMS are also included for the following:

(1) a labeled oligonucleotide that specifically hybridizes to an **HIV-1** sequence derived from gag or pol sequences, having one of the

sequences fully defined in the specification, and
(2) detecting **HIV-1** in a biological sample, comprising mixing the sample with two or more of the claimed amplification oligomers that specifically amplify at least one **HIV-1** target sequence within gag and a pol sequence which is a protease or reverse transcriptase sequence, amplifying the target, and detecting the amplified product.
USE - The invention is used to diagnose **HIV-1** infection.
Dwg.0/2

L4 ANSWER 4 OF 5 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2002-292273 [33] WPIDS
DNC C2002-085928
TI New nucleic acid oligomer, useful for detecting selected regions of gag and pol genes of human immune deficiency virus, particularly for assessing drug resistance.
DC B04 D16
IN BABOLA, O; BRENTANO, S T; TRAN, N; VERNET, G; **YANG, Y Y**
PA (INMR) BIOMERIEUX SA; (GENP-N) GEN-PROBE INC
CYC 95
PI WO 2002020852 A1 20020314 (200233)* EN 83p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2000071042 A 20020322 (200251)
EP 1315839 A1 20030604 (200337) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
ADT WO 2002020852 A1 WO 2000-US24117 20000901; AU 2000071042 A AU 2000-71042 20000901, WO 2000-US24117 20000901; EP 1315839 A1 EP 2000-959778 20000901, WO 2000-US24117 20000901
FDT AU 2000071042 A Based on WO 2002020852; EP 1315839 A1 Based on WO 2002020852
PRAI WO 2000-US24117 20000901
AB WO 200220852 A UPAB: 20020524
NOVELTY - A nucleic acid oligomer (I) for amplifying part of the gag or pol genes of human immune deficiency virus (**HIV**)-**1** is any of 54 sequences (S5-S22 and S33-S68) fully defined in the specification, where (I) may include one or more of 2'-O-methoxy, peptide-nucleic acid, phosphorothioate and/or methylphosphonate linkages, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) a mixture of (I) to amplify a first and second gag sequence and a protease sequence;
(2) a detectably labeled oligonucleotides (LON) comprising S23-S29 that hybridize specifically to **HIV-1** gag and pol sequences;
(3) detecting **HIV-1** using the mixtures of (I).
USE - (I) are used to detect regions of the gag and pol genes, especially regions associated with drug resistance, but also for identifying genetic subtypes of the virus.
ADVANTAGE - Many different segments of the viral genome can be amplified in a single reaction vessel, minimizing the number of reactions that have to be done and avoiding risks of a false negative result if one region is not properly amplified.
Dwg.0/2

L4 ANSWER 5 OF 5 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2001-147200 [15] WPIDS
DNC C2001-043540
TI Detecting **HIV-1** nucleic acids in biological samples useful for diagnosing **HIV-1** infection involves using nucleic acid capture oligomers, amplification oligomers and probe oligomers.

```
IN   BEE, G G; GIACHETTI, C; KOLK, D P; MCDONOUGH, S H; YANG, Y Y; KOLK, D
PA   (BEEG-I) BEE G G; (GENP-N) GEN-PROBE INC; (GIAC-I) GIACHETTI C; (KOLK-I)
     KOLK D P; (MCDO-I) MCDONOUGH S H; (YANG-I) YANG Y Y
CYC  23
PI   WO 2001004361 A2 20010118 (200115)* EN   60p
        RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
         W: AU CA JP US
     AU 2000060794 A  20010130 (200127)
     EP 1187939    A2 20020320 (200227)  EN
         R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
     JP 2003504077 W  20030204 (200320)        97p
     US 6623920    B1 20030923 (200364)
ADT  WO 2001004361 A2 WO 2000-US18685 20000707; AU 2000060794 A AU 2000-60794
     20000707; EP 1187939 A2 EP 2000-947134 20000707, WO 2000-US18685 20000707;
     JP 2003504077 W WO 2000-US18685 20000707, JP 2001-509559 20000707; US
     6623920 B1 Provisional US 1999-143072P 19990709, US 2000-611627 20000707
FDT  AU 2000060794 A Based on WO 2001004361; EP 1187939 A2 Based on WO
     2001004361; JP 2003504077 W Based on WO 2001004361
PRAI US 1999-143072P  19990709; US 2000-611627   20000707
AB   WO 200104361 A UPAB: 20010317
     NOVELTY - An oligomer comprising one of 57 nucleotide sequences ranging
     from 17 to 57 nucleotides in length described in the specification is new.
          DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for:
          (1) a labelled oligomer comprising:
          (a) one of 17 nucleotide sequences described in the specification;
     and
          (b) a detectable label joined directly or indirectly to the base
     sequence.
          (2) detecting HIV-1 RNA in a biological sample, comprising:
          (a) providing a biological sample containing HIV-1 RNA;
          (b) contacting the biological sample with at least one capture
     oligomer comprising a base sequence that hybridizes specifically to a
     target region in the long terminal repeat (LTR) or pol sequences of
     HIV-1 RNA, thus forming a capture oligomer:HIV-1 complex;
          (c) separating the capture oligomer:HIV-1 RNA complex from the
     biological sample;
          (d) then amplifying the LTR or pol sequences, or a cDNA made from it
     using a nucleic acid polymerase in vitro to produce an amplified product;
     and
          (e) detecting the amplified product using a labeled detection probe
     that hybridizes specifically with the amplified product.
          USE - Detecting HIV-1, RNA biological sample (claimed) derived
     from humans preferably in blood, serum or plasma. The nucleic acid
     sequences are useful for capturing, amplifying, detecting HIV-1
     nucleic acid present in a biological sample. The methods are thus
     important for diagnosis of HIV-1 infection and for screening blood and
     blood products that may contain infectious virus, to prevent infecting
     individuals through transfusion with infected blood or plasma. The
     screening is also important to prevent HIV-1 contamination is
     blood-derived therapeutics.
          DESCRIPTION OF DRAWING(S) - The figure shows the detection of
     HIV-1 nucleic acid target region using two capture oligomers, two
     non-T7 primers and two labeled probes.
     Dwg.3/4


=> file medline
COST IN U.S. DOLLARS                               SINCE FILE     TOTAL
                                                        ENTRY   SESSION
FULL ESTIMATED COST                                     28.29     43.59

FILE 'MEDLINE' ENTERED AT 19:03:12 ON 10 MAR 2004

FILE LAST UPDATED: 9 MAR 2004 (20040309/UP).  FILE COVERS 1953 TO DATE.
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for details.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2004 vocabulary. See http://www.nlm.nih.gov/mesh/ and http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a description of changes.

This file contains CAS Registry Numbers for easy and accurate substance identification.

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=> e yang y y/au
E1            2      YANG Y WENDY/AU
E2           32      YANG Y X/AU
E3          105 --> YANG Y Y/AU
E4            1      YANG Y Y YI YAN/AU
E5           91      YANG Y Z/AU
E6            4      YANG YA/AU
E7            1      YANG YA CHEN/AU
E8            3      YANG YA CHIEN/AU
E9            2      YANG YA CHIN/AU
E10           1      YANG YA JU/AU
E11           2      YANG YA LI/AU
E12           1      YANG YA MING/AU

=> s e3
L5          105 "YANG Y Y"/AU

=> s l5 and (HIV-1 or HIV-2 or HIV)
         134464 HIV
        3179457 1
          42420 HIV-1
                  (HIV(W)1)
         134464 HIV
        2772183 2
           3675 HIV-2
                  (HIV(W)2)
         134464 HIV
L6            1 L5 AND (HIV-1 OR HIV-2 OR HIV)

=> d l6,cbib,ab
```

L6 ANSWER 1 OF 1 MEDLINE on STN
94295994. PubMed ID: 8024197. Cationic liposomes for direct gene transfer in therapy of cancer and other diseases. Farhood H; Gao X; Son K; **Yang Y Y**; Lazo J S; Huang L; Barsoum J; Bottega R; Epand R M. (Department of Pharmacology, University of Pittsburgh School of Medicine, Pennsylvania 15261.) Annals of the New York Academy of Sciences, (1994 May 31) 716 23-34; discussion 34-5. Ref: 29. Journal code: 7506858. ISSN: 0077-8923. Pub. country: United States. Language: English.

AB Cationic liposomes can mediate efficient delivery of DNA and DNA/protein complex to mammalian cells in vitro and in vivo. Cationic cholesterol derivatives mixed with phosphatidylethanolamine and sonicated to form small unilamellar vesicles can complex with DNA and mediate the entry into the cytosol from the endosome compartment. One of the liposome formulations, DC-Chol liposomes, is used in a gene therapy clinical trial for melanoma. Recently, we exploited these cationic liposomes for the delivery of trans-activating protein factors to regulate and control the expression of delivered transgenes in a protein dose-dependent manner. Bacteriophage T7 RNA polymerase was co-delivered with a reporter gene under the control of T7 promoter to allow cytoplasmic expression of the gene. Human immunodeficiency virus-1 transactivating protein was also codelivered with a reporter gene under the control of **HIV-1** long terminal repeat. Finally, human tumor cells selected for cis-platin resistance or isolated from patients who have failed cis-platin therapy are highly transfectable with cationic liposomes. These results suggest a

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=> e burrell terrie a/au
E1            1      BURRELL T M/AU
E2            1      BURRELL TERRIE/AU
E3            0 --> BURRELL TERRIE A/AU
E4            3      BURRELL W/AU
E5            2      BURRELL W C/AU
E6            5      BURRELL W E/AU
E7            2      BURRELL Z/AU
E8            2      BURRELL Z L/AU
E9            9      BURRELL Z L JR/AU
E10           4      BURRELLI R/AU
E11           1      BURRELLO C/AU
E12           3      BURRELLO L C/AU

=> e e1
E1            1      BURRELL T/AU
E2            1      BURRELL T A/AU
E3            1 --> BURRELL T M/AU
E4            1      BURRELL TERRIE/AU
E5            3      BURRELL W/AU
E6            2      BURRELL W C/AU
E7            5      BURRELL W E/AU
E8            2      BURRELL Z/AU
E9            2      BURRELL Z L/AU
E10           9      BURRELL Z L JR/AU
E11           4      BURRELLI R/AU
E12           1      BURRELLO C/AU

=> s e2
L7            1 "BURRELL T A"/AU

=> d l7,cbib,ab
```

L7 ANSWER 1 OF 1 MEDLINE on STN

92113032. PubMed ID: 1730783. Regulation of NF-kappa B activity in murine macrophages: effect of bacterial lipopolysaccharide and phorbol ester. Vincenti M P; **Burrell T A**; Taffet S M. (Department of Microbiology and Immunology, S.U.N.Y. Health Science Center, Syracuse 13210.) Journal of cellular physiology, (1992 Jan) 150 (1) 204-13. Journal code: 0050222. ISSN: 0021-9541. Pub. country: United States. Language: English.

AB Nuclear factor kappa-B (NF-kappa B) has been shown to play an important role in LPS-mediated induction of several genes in macrophages. Several studies have implicated protein kinase C (PKC) or cAMP-dependent protein kinase in the regulation of NF-kappa B activity. In this study we have investigated the mechanism of NF-kappa B induction in murine macrophages. A chloramphenicol acetyl transferase (CAT) expression vector containing multiple copies of the TNF-alpha NF-kappa B element was transfected into the RAW264 macrophage-like cell line and assessed for inducible CAT activity. LPS treatment of the transfected cells resulted in a significant induction of CAT activity. CAT activity was not induced by treatment with phorbol myristate acetate (PMA) or the cAMP analogue 8-bromo cAMP. To further study NF-kappa B induction, nuclear extracts were prepared from RAW264 cells. Extracts from RAW264 cells that were treated from 30 min to 2 hr with LPS had a significant increase in NF-kappa B binding activity as determined by the electrophoresis mobility shift assay (EMSA). Treatment of these cells from 30 min to 2 hr with PMA did not result in such binding activity. U.V. crosslinking analysis of the DNA-binding activity confirmed these results and indicated that LPS induced a 55 KD DNA-binding protein. Induction of this NF-kappa B binding activity was not inhibited by pretreatment with the PKC inhibitor H-7. H-7 did inhibit induction of TPA responsive element binding by either LPS or PMA. Prolonged exposure to phorbol ester, a treatment which down-regulates PKC, had no effect on LPS induction of NF-kappa B activity

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     in these cells. These results suggest that the induction of NF-kappa B in
     macrophages by LPS is independent of PKC.


=> file uspatful
COST IN U.S. DOLLARS                                  SINCE FILE     TOTAL
                                                           ENTRY   SESSION
FULL ESTIMATED COST                                         1.20     44.79

FILE 'USPATFULL' ENTERED AT 19:04:17 ON 10 MAR 2004
CA INDEXING COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 9 Mar 2004 (20040309/PD)
FILE LAST UPDATED: 9 Mar 2004 (20040309/ED)
HIGHEST GRANTED PATENT NUMBER: US6704933
HIGHEST APPLICATION PUBLICATION NUMBER: US2004045070
CA INDEXING IS CURRENT THROUGH 9 Mar 2004 (20040309/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 9 Mar 2004 (20040309/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Dec 2003
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Dec 2003

>>>  USPAT2 is now available.  USPATFULL contains full text of the     <<<
>>>  original, i.e., the earliest published granted patents or         <<<
>>>  applications.  USPAT2 contains full text of the latest US         <<<
>>>  publications, starting in 2001, for the inventions covered in     <<<
>>>  USPATFULL.  A USPATFULL record contains not only the original     <<<
>>>  published document but also a list of any subsequent              <<<
>>>  publications.  The publication number, patent kind code, and      <<<
>>>  publication date for all the US publications for an invention     <<<
>>>  are displayed in the PI (Patent Information) field of USPATFULL   <<<
>>>  records and may be searched in standard search fields, e.g., /PN, <<<
>>>  /PK, etc.                                                         <<<

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>>>  through the new cluster USPATALL.  Type FILE USPATALL to          <<<
>>>  enter this cluster.                                               <<<
>>>                                                                    <<<
>>>  Use USPATALL when searching terms such as patent assignees,       <<<
>>>  classifications, or claims, that may potentially change from      <<<
>>>  the earliest to the latest publication.                           <<<

This file contains CAS Registry Numbers for easy and accurate
substance identification.

=> s (oligonucleotide/clm or primer/clm)
          9615 OLIGONUCLEOTIDE/CLM
         10293 PRIMER/CLM
L8       17820 (OLIGONUCLEOTIDE/CLM OR PRIMER/CLM)

=> s l8 and oligonucleotide/clm
          9615 OLIGONUCLEOTIDE/CLM
L9        9615 L8 AND OLIGONUCLEOTIDE/CLM

=> s l9 and analog?
        534500 ANALOG?
L10       7157 L9 AND ANALOG?

=> s l10 and analog?/clm
         67615 ANALOG?/CLM
L11        789 L10 AND ANALOG?/CLM

=> s l11 and methoxy/clm
         31333 METHOXY/CLM
L12         52 L11 AND METHOXY/CLM

=> s l12 and chemiluminescent/clm
```

L13 8 L12 AND CHEMILUMINESCENT/CLM

=> d l13,cbib,1-8

L13 ANSWER 1 OF 8 USPATFULL on STN
2003:237676 Polynucleotide probes for detection and quantitation of candida species.
Hogan, James J., Coronado, CA, UNITED STATES
Gordon, Patricia C., Spring Valley, CA, UNITED STATES
Gen-Probe Incorporated (U.S. corporation)
US 2003165833 A1 20030904
APPLICATION: US 2001-846797 A1 20010501 (9)
PRIORITY: US 2000-201249P 20000501 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 2 OF 8 USPATFULL on STN
2003:140412 Single nucleotide amplification and detection by polymerase.
Nelson, John, Neshanic Station, NJ, UNITED STATES
Fuller, Carl, Berkeley Heights, NJ, UNITED STATES
Sood, Anup, Flemington, NJ, UNITED STATES
Kumar, Shiv, Belle Mead, NJ, UNITED STATES
US 2003096253 A1 20030522
APPLICATION: US 2002-113025 A1 20020401 (10)
PRIORITY: US 2001-315798P 20010829 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 3 OF 8 USPATFULL on STN
2003:112878 Terminal-phosphate-labeled nucleotides and methods of use.
Nelson, John, Neshanic Station, NJ, UNITED STATES
Fuller, Carl, Berkeley Heights, NJ, UNITED STATES
Sood, Anup, Flemington, NJ, UNITED STATES
Kumar, Shiv, Belle Mead, NJ, UNITED STATES
US 2003077610 A1 20030424
APPLICATION: US 2002-113030 A1 20020401 (10)
PRIORITY: US 2001-315798P 20010829 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 4 OF 8 USPATFULL on STN
2002:314651 Compositions and methods for detecting human immunodeficiency virus 2 (HIV-2).
Yang, Yeasing Y., San Diego, CA, UNITED STATES
Burrell, Terrie A., San Diego, CA, UNITED STATES
US 2002177127 A1 20021128
APPLICATION: US 2001-1407 A1 20011022 (10)
PRIORITY: US 2000-242620P 20001023 (60)
US 2001-280058P 20010330 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 5 OF 8 USPATFULL on STN
2002:88211 Polynucleotide probes for detection and quantitation of staphylococcus.
Hogan, James J., Coronado, CA, United States
Gordon, Patricia, San Diego, CA, United States
Gen-Probe Incorporated, San Diego, CA, United States (U.S. corporation)
US 6376186 B1 20020423
APPLICATION: US 2000-565241 20000503 (9)
PRIORITY: US 1999-132409P 19990503 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 6 OF 8 USPATFULL on STN

2001:[illegible] Polynucleotide probes for detection and quantitation of
    actinomycetes.
    Hogan, James J., Coronado, CA, United States
    Gordon, Patricia, San Diego, CA, United States
    Gen-Probe Incorporated, San Diego, CA, United States (U.S. corporation)
    US 6235484 B1 20010522
    APPLICATION: US 2000-565596 20000503 (9)
    PRIORITY: US 1999-132412P 19990503 (60)
    DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 7 OF 8 USPATFULL on STN
1999:170726 Base-protected nucleotide **analogs** with protected thiol groups.
    Hanna, Michelle M., Norman, OK, United States
    The Board of Regents of the University of Oklahoma, Norman, OK, United
    States (U.S. corporation)
    US 6008334 19991228
    APPLICATION: US 1997-899022 19970723 (8)
    PRIORITY: US 1996-22573P 19960724 (60)
    DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 8 OF 8 USPATFULL on STN
97:15952 Assays using chemiluminescent, enzymatically cleavable substituted
    1,2-dioxetanes and kits therefor.
    Bronstein, Irena Y., Newton, MA, United States
    Tropix, Inc., Bedford, MA, United States (U.S. corporation)
    US 5605795 19970225
    APPLICATION: US 1994-255795 19940607 (8)
    DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l13,cbib,ab,clm,kwic,5-8

L13 ANSWER 5 OF 8 USPATFULL on STN
2002:88211 Polynucleotide probes for detection and quantitation of
    staphylococcus.
    Hogan, James J., Coronado, CA, United States
    Gordon, Patricia, San Diego, CA, United States
    Gen-Probe Incorporated, San Diego, CA, United States (U.S. corporation)
    US 6376186 B1 20020423
    APPLICATION: US 2000-565241 20000503 (9)
    PRIORITY: US 1999-132409P 19990503 (60)
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB   Polynucleotide probes and accessory helper oligonucleotides useful for detecting bacteria that are members of the genus Staphylococcus. The hybridization probes are highly specific for Staphylococcal bacteria and do not cross-hybridize with the rRNA or rDNA of numerous other bacterial and fungal species.

CLM   What is claimed is:
        1. A probe composition for detecting nucleic acids of bacteria that are members of the Staphylococcus genus, comprising: an **oligonucleotide** probe that hybridizes under a high stringency condition to a Staphylococcal 16S rRNA or rDNA to form a detectable probe:target duplex, wherein said **oligonucleotide** probe has the length and sequence of SEQ ID NO:1 or the complement thereof, and optionally a non-complementary sequence that does not hybridize to said Staphylococcal 16S rRNA or rDNA, and wherein under said hybridization condition said **oligonucleotide** probe hybridizes to nucleic acids present in Staphylococcus aureus, Staphylococcus cohnii, Staphylococus delphi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus hyicus, Staphylococcus intermedius, Staphylococcus saprophyticus, Staphylococcus simulan and Staphylococcus warneri.

2. The probe composition of claim 1, wherein the **oligonucleotide** probe comprises DNA.

3. The probe composition of claim 1, wherein said high stringency condition comprises 0.48 M sodium phosphate buffer, 0.1% sodium dodecyl sulfate, and 1 mM each of EDTA and EGTA.

4. The probe composition of claim 1 wherein said high stringency condition comprises 0.6 M LiCl, 1% lithium lauryl sulfate, 60 mM lithium succinate and 10 mM each of EDTA and EGTA.

5. The probe composition of claim 1, wherein said **oligonucleotide** probe has the length and sequence of SEQ ID NO:1.

6. The probe composition of claim 1, wherein said **oligonucleotide** probe further comprises a detectable label.

7. The probe composition of claim 5, wherein said **oligonucleotide** probe further comprises a detectable label.

8. The probe composition of claim 6 or claim 7 wherein the detectable label is a **chemiluminescent** label or a radioactive label.

9. The probe composition of claim 8, wherein the detectable label is a **chemiluminescent** label, and wherein the chemiluminescenct label is an acridinium ester.

10. The probe composition of claim 8, further comprising at least one helper **oligonucleotide**.

11. The probe composition of claim 10, wherein said at least one helper **oligonucleotide** includes at least one nucleotide **analog**.

12. The probe composition of claim 11, wherein said at least one nucleotide **analog** comprises a ribose moiety having a **methoxy** group disposed at the 2' position.

13. The probe composition of claim 10, wherein said at least one helper **oligonucleotide** has a sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3.

14. A method for detecting the presence of Staphylococcus bacteria in a test sample, comprising the steps of: (a) providing to said test sample a probe composition that includes an **oligonucleotide** probe that hybridizes under a high stringency condition to a Staphylococcal 16S rRNA or rDNA to form a detectable probe:target duplex, wherein said **oligonucleotide** probe has the length and sequence of SEQ ID NO:1 or the complement thereof, and optionally a non-complementary sequence that does not hybridize to said Staphylococcal 16S rRNA or rDNA, and wherein under said hybridization condition said **oligonucleotide** probe hybridizes to nucleic acids present in Staphylococcus aureus, Staphylococcus cohnii, Staphylococcus delphi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus hyicus, Staphylococcus intermedius, Staphylococcus saprophyticus, Staphylococcus simulan and Staphylococcus warneri; (b) hybridizing under said high stringency condition any nucleic acids from Staphylococcus bacteria that are present in the test sample with said probe composition to form a probe:target duplex; and (c) detecting said probe:target duplex of (b) as an indicator of the presence of Staphylococcus bacteria in the test sample.

15. The method of claim 14, wherein said test sample may comprise bacteria, and wherein before step (a) there is a step for releasing nucleic acids from any bacteria that may be present in said test sample.

16. The method of claim 14, wherein said test sample is a lysate.

17. The method of claim 14, wherein said high stringency condition comprises 0.48 M sodium phosphate buffer, 0.1% sodium dodecyl sulfate, and 1 mM each of EDTA and EGTA.

18. The method of claim 14, wherein said high stringency condition comprises 0.6 M LiCl, 1% lithium lauryl sulfate, 60 mM lithium succinate and 10 mM each of EDTA and EGTA.

19. The method of claim 14, wherein the **oligonucleotide** probe has the length and sequence of SEQ ID NO:1.

20. The method of claim 19, wherein the **oligonucleotide** probe comprises a detectable label.

21. The method of claim 20, wherein the detectable label is an acridinium ester, and wherein the detecting step comprises performing luminometry to detect any of said probe:target duplex of (b).

22. The method of claim 20, wherein said probe composition further comprises at least one helper **oligonucleotide** that facilitates formation of the probe:target duplex of (b).

23. The method of claim 22, wherein said at least one helper **oligonucleotide** is selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3.

24. A kit for detecting in a test sample the presence of nucleic acids from bacteria that are members of the Staphylococcus genus, comprising: (a) a probe composition that includes an **oligonucleotide** probe that hybridizes under a high stringency condition to a Staphylococcal 16S rRNA or rDNA to form a detectable probe:target duplex, wherein said **oligonucleotide** probe has the length and sequence of SEQ ID NO:1 or the complement thereof, and optionally a non-complementary sequence that does not hybridize to said Staphylococcal 16S rRNA or rDNA, and wherein under said hybridization condition said **oligonucleotide** probe hybridizes to nucleic acids present in Staphylococcus aureus, Staphylococcus cohnii, Staphylococcus delphi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus hyicus, Staphylococcus intermedius, Staphylococcus saprophyticus, Staphylococcus simulan and Staphylococcus warneri; and (b) printed instructions specifying, in order of implementation, steps to be followed for detecting nucleic acids from bacteria that are members of the Staphylococcus genus by detecting a complex between the **oligonucleotide** probe and a Staphylococcus nucleic acid target, wherein said probe composition and said printed instructions are in packaged combination.

25. The probe composition of claim 1, wherein said **oligonucleotide** probe includes said non-complementary sequence, and wherein said non-complementary sequence is selected from the group consisting of a promoter sequence, a restriction endonuclease recognition site, a sequence that confers a secondary structure, and a sequence that confers a tertiary structure.

SUMM . . . of EDTA and EGTA. The oligonucleotide probe may be made of DNA, but also may include at least one nucleotide **analog**. For example, the nucleotide **analog** may include a methoxy group at the 2' position of a ribose moiety. In one embodiment the invented oligonucleotide probe. . .

SUMM . . . formation of the detectable probe:target duplex under high stringency hybridization conditions. These helper oligonucleotides may include at least one nucleotide **analog**, such as a ribose moiety having a methoxy group disposed at the 2' position. In a highly preferred embodiment of. . .

SUMM . . . sugar is a deoxyribose. For a 5' nucleotide, the sugar contains a hydroxyl group (--OH) at the 5'-carbon-5. The term also includes **analogs** of such subunits, such as a methoxy group at the 2' position of the ribose (OMe). As used herein, methoxy. . .

SUMM . . . hydrogen bonding with a nucleotide, and would exclude units having as a component one of the five nucleotide bases or **analogs** thereof.

SUMM Nitrogenous base **analogs** also may be components of oligonucleotides in accordance with the invention.

DETD . . . complement of SEQ ID NO:10. Preferred oligonucleotide sequences include RNA and DNA equivalents, and may include at least one nucleotide **analog**.

DETD . . . "helper oligonucleotides" or simply "oligonucleotides" embrace polymers of native nucleotides as well as polymers that include at least one nucleotide **analog**.

DETD Backbone-modified oligonucleotides, such as those having phosphorothioate or methylphosphonate groups, are examples of **analogs** that can be used in conjunction with oligonucleotides of the present invention. These modifications render the oligonucleotides resistant to the nucleolytic activity of certain polymerases or to nuclease enzymes. Other **analogs** that can be incorporated into the structures of the oligonucleotides disclosed herein include peptide nucleic acids, or "PNAs." The PNAs. . .

1. A probe composition for detecting nucleic acids of bacteria that are members of the Staphylococcus genus, comprising: an **oligonucleotide** probe that hybridizes under a high stringency condition to a Staphylococcal 16S rRNA or rDNA to form a detectable probe:target duplex, wherein said **oligonucleotide** probe has the length and sequence of SEQ ID NO:1 or the complement thereof, and optionally a non-complementary sequence that does not hybridize to said Staphylococcal 16S rRNA or rDNA, and wherein under said hybridization condition said **oligonucleotide** probe hybridizes to nucleic acids present in Staphylococcus aureus, Staphylococcus cohnii, Staphylococus delphi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus. . .

2. The probe composition of claim 1, wherein the **oligonucleotide** probe comprises DNA.

5. The probe composition of claim 1, wherein said **oligonucleotide** probe has the length and sequence of SEQ ID NO:1.

6. The probe composition of claim 1, wherein said **oligonucleotide** probe further comprises a detectable label.

7. The probe composition of claim 5, wherein said **oligonucleotide** probe further comprises a detectable label.

8. The probe composition of claim 6 or claim 7 wherein the detectable label is a **chemiluminescent** label or a radioactive label.

9. The probe composition of claim 8, wherein the detectable label is a **chemiluminescent** label, and wherein the chemiluminescenct label is an acridinium ester.

10. The probe composition of claim 8, further comprising at least one helper **oligonucleotide**.

11. The probe composition of claim 10, wherein said at least one helper **oligonucleotide** includes at least one nucleotide **analog**.

12. The probe composition of claim 11, wherein said at least one nucleotide **analog** comprises a ribose moiety having a **methoxy** group disposed at the 2' position.

13. The probe composition of claim 10, wherein said at least one helper **oligonucleotide** has a sequence selected from the group consisting of

SEQ ID NO:2 and SEQ ID NO:3.

. . . in a test sample, comprising the steps of: (a) providing to said test sample a probe composition that includes an **oligonucleotide** probe that hybridizes under a high stringency condition to a Staphylococcal 16S rRNA or rDNA to form a detectable probe:target duplex, wherein said **oligonucleotide** probe has the length and sequence of SEQ ID NO:1 or the complement thereof, and optionally a non-complementary sequence that does not hybridize to said Staphylococcal 16S rRNA or rDNA, and wherein under said hybridization condition said **oligonucleotide** probe hybridizes to nucleic acids present in Staphylococcus aureus, Staphylococcus cohnii, Staphylococcus delphi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus. . . .
19. The method of claim 14, wherein the **oligonucleotide** probe has the length and sequence of SEQ ID NO:1.

20. The method of claim 19, wherein the **oligonucleotide** probe comprises a detectable label.

22. The method of claim 20, wherein said probe composition further comprises at least one helper **oligonucleotide** that facilitates formation of the probe:target duplex of (b).

23. The method of claim 22, wherein said at least one helper **oligonucleotide** is selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3.

. . . of nucleic acids from bacteria that are members of the Staphylococcus genus, comprising: (a) a probe composition that includes an **oligonucleotide** probe that hybridizes under a high stringency condition to a Staphylococcal 16S rRNA or rDNA to form a detectable probe:target duplex, wherein said **oligonucleotide** probe has the length and sequence of SEQ ID NO:1 or the complement thereof, and optionally a non-complementary sequence that does not hybridize to said Staphylococcal 16S rRNA or rDNA, and wherein under said hybridization condition said **oligonucleotide** probe hybridizes to nucleic acids present in Staphylococcus aureus, Staphylococcus cohnii, Staphylococcus delphi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus. . . . followed for detecting nucleic acids from bacteria that are members of the Staphylococcus genus by detecting a complex between the **oligonucleotide** probe and a Staphylococcus nucleic acid target, wherein said probe composition and said printed instructions are in packaged combination.

25. The probe composition of claim 1, wherein said **oligonucleotide** probe includes said non-complementary sequence, and wherein said non-complementary sequence is selected from the group consisting of a promoter sequence,. . . .

L13 ANSWER 6 OF 8 USPATFULL on STN
2001:75137 Polynucleotide probes for detection and quantitation of actinomycetes.
Hogan, James J., Coronado, CA, United States
Gordon, Patricia, San Diego, CA, United States
Gen-Probe Incorporated, San Diego, CA, United States (U.S. corporation)
US 6235484 B1 20010522
APPLICATION: US 2000-565596 20000503 (9)
PRIORITY: US 1999-132412P 19990503 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Polynucleotide probes and accessory helper oligonucleotides useful for detecting the subset of High (G+C) subset of Gram-positive bacteria known as the "Actinomycetes." The hybridization probes are highly specific for the Actinomycetes and do not cross-hybridize with the rRNA or rDNA of numerous other bacterial and fungal species.

1. An **oligonucleotide** probe that specifically hybridizes to an Actinomycetes nucleic acid target region corresponding to E. coli 235 rRNA nucleotide positions 1986-2064 under a high stringency hybridization condition to form a detectable probe:target duplex, said **oligonucleotide** probe having a length of up to 100 nucleotides and comprising at least 17 contiguous nucleotides contained within the sequence of SEQ ID NO:10 or the complement thereof.

2. The **oligonucleotide** probe of claim 1, wherein said probe comprises at least 25 contiguous nucleotides contained within the sequence of SEQ ID NO:10.

3. The **oligonucleotide** probe of claim 2, wherein said probe comprises at least 29 contiguous nucleotides contained within the sequence of SEQ ID NO:10.

4. The **oligonucleotide** probe of claim 1, wherein the high stringency hybridization condition is provided by 0.48 M sodium phosphate buffer, 0.1% sodium dodecyl sulfate, 1 mM each of EDTA and EGTA.

5. The **oligonucleotide** probe of claim 1, wherein the high stringency hybridization condition is provided by 0.6 M LiCl, 1% lithium lauryl sulfate, 60 mM lithium succinate and 10 mM each of EDTA and EGTA.

6. The **oligonucleotide** probe of claim 1, wherein said **oligonucleotide** probe comprises DNA.

7. The **oligonucleotide** probe of claim 1, wherein said **oligonucleotide** probe comprises at least one nucleotide **analog**.

8. The **oligonucleotide** probe of claim 7, wherein said at least one nucleotide **analog** comprises a **methoxy** group at the 2' position of a ribose moiety.

9. The **oligonucleotide** probe of claim 1, wherein said **oligonucleotide** probe has a sequence selected from the group consisting of SEQ ID NO:1 or the complement thereof, SEQ ID NO:2 or the complement thereof, and SEQ ID NO:3 or the complement thereof.

10. The **oligonucleotide** of claim 9, wherein said sequence is given by SEQ ID NO:2 or SEQ ID NO:3, said **oligonucleotide** being a helper **oligonucleotide**.

11. The **oligonucleotide** probe of claim 9, further comprising a detectable label.

12. The **oligonucleotide** probe of claim 11, wherein the detectable label is a **chemiluminescent** label or a radiolabel.

13. The **oligonucleotide** probe of claim 9, wherein said sequence is given by SEQ ID NO:1.

14. The **oligonucleotide** probe of claim 13, wherein said **oligonucleotide** probe further comprises a detectable label.

15. The **oligonucleotide** probe of claim 14, wherein the detectable label is an acridinium ester.

16. A probe composition for detecting nucleic acids of Actinomycetes bacteria, comprising: an **oligonucleotide** probe that hybridizes under a high stringency condition to an Actinomycetes nucleic acid target region corresponding to E. coli 23S rRNA nucleotide positions 1986 - 2064 to form a detectable target:probe duplex, wherein said **oligonucleotide** probe has a length of up to 100 nucleotide bases and comprises at least 17 contiguous nucleotides contained within the sequence of SEQ ID NO: 10

or the complement thereof, and wherein under said hybridization condition said **oligonucleotide** probe specifically hybridizes to nucleic acids present in Corynebacterium aquaticum, Corynebacterium jeikieum, Corynebacterium xerosis, Micrococcus luteus, Propionibacterium acnes, Mycobacterium chelonae, Mycobacterium terrae, Mycobacterium intracellulare, Mycobacterium simiae, Mycobacterium avium, Mycobacterium scrofulaceum, Mycobacterium gordonae, Mycobacterium kansasii, Mycobacterium smegatis, Mycobacteriumfortuitum, Mycobacterium gastri, Mycobacterium xenopi, Mycobacterium marinum and Mycobacterium phlei.

17. The probe composition of claim 1, wherein said **oligonucleotide** probe has a length of up to 100 nucleotide bases and comprises at least 25 contiguous nucleotides contained within the sequence of SEQ ID NO:10 or the complement thereof.

18. The probe composition of claim 16, wherein the **oligonucleotide** probe comprises DNA.

19. The probe composition of claim 16, wherein said high stringency condition is provided by 0.48 M sodium phosphate buffer, 0.1% sodium dodecyl sulfate, 1 mM each of EDTA and EGTA.

20. The probe composition of claim 16, wherein said high stringency condition is provided by 0.6 M LiCl, 1% lithium lauryl sulfate, 60 mM lithium succinate and 10 mM each of EDTA and EGTA.

21. The probe composition of claim 16, wherein said **oligonucleotide** probe comprises the sequence of SEQ ID NO:1 or the complement thereof.

22. The probe composition of claim 16, wherein the length of said **oligonucleotide** probe is up to 60 bases.

23. The probe composition of claim 16, wherein said **oligonucleotide** probe has the length and sequence of SEQ ID NO:1.

24. The probe composition of claim 16, wherein said **oligonucleotide** probe further comprises a detectable label.

25. The probe composition of claim 22, wherein said **oligonucleotide** probe further comprises a detectable label.

26. The probe composition of claim 23, wherein said **oligonucleotide** probe further comprises a detectable label.

27. The probe composition of any one of claims 24, 25 or 26 wherein the detectable label is a **chemiluminescent** label or a radiolabel.

28. The probe composition of claim 27, wherein the **chemiluminescent** label is an acridinium ester.

29. The probe composition of claim 27, further comprising at least one helper **oligonucleotide** that facilitates formation of the detectable probe:target duplex under said hybridization conditions.

30. The probe composition of claim 29, wherein said at least one helper **oligonucleotide** includes at least one nucleotide **analog**.

31. The probe composition of claim 30, wherein said at least one nucleotide **analog** comprises a ribose moiety having a **methoxy** group disposed at the 2' position.

32. The probe composition of claim 29, wherein said at least one helper **oligonucleotide** has a sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3.

33. A method for detecting the presence of Actinomycetes in a test

sample, comprising the steps of: (a) providing to said test sample a probe composition comprising an **oligonucleotide** probe that hybridizes under a high stringency condition to an Actinomycetes nucleic acid target region corresponding to E. coli 23S rRNA nucleotide positions 1986-2064 to form a detectable target:probe duplex, said **oligonucleotide** probe having a length of up to 100 nucleotide bases and comprising at least 25 contiguous nucleotides contained within the sequence of SEQ ID NO:10 or the complement thereof, and wherein under said hybridization condition said **oligonucleotide** probe specifically hybridizes to nucleic acids present in Corynebacterium aquaticum, Corynebacterium jeikieum, Corynebacterium xerosis, Micrococcus luteus, Propionibacterium acnes, Mycobacterium chelonae, Mycobacterium terrae, Mycobacterium intracellulare, Mycobacterium simiae, Mycobacterium avium, Mycobacterium scroffulaceum, Mycobacterium gordonae, Mycobacterium kansasii, Mycobacterium smegatis, Mycobacteriumfortuitum, Mycobacterium gastri, Mycobacterium xenopi, Mycobacterium marinum and Mycobacterium phlei; (b) hybridizing under high stringency conditions any Actinomycetes nucleic acid that may be present in the test sample with said probe composition to form a probe:target duplex; and (c) detecting said probe:target duplex as an indicator of the presence of Actinomycetes in the test sample.

34. The method of claim 33, wherein said test sample may comprise bacteria, and wherein before step (a) there is a step for releasing nucleic acid from any bacteria that may be present in said test sample.

35. The method of claim 33, wherein said test sample is a lysate.

36. The method of claim 33, wherein said high stringency hybridization conditions are provided by 0.48 M sodium phosphate buffer, 0.1% sodium dodecyl sulfate, 1 mM each of EDTA and EGTA.

37. The method of claim 33, wherein said high stringency hybridization conditions are provided by 0.6 M LiCl, 1% lithium lauryl sulfate, 60 mM lithium succinate and 10 mM each of EDTA and EGTA.

38. The method of claim 33, wherein the **oligonucleotide** probe has the length and sequence of SEQ ID NO: 1.

39. The method of claim 38, wherein the **oligonucleotide** probe comprises a detectable label.

40. The method of claim 39, wherein the detectable label is an acridinium ester, and wherein the detecting step comprises performing luminometry to detect any of said probe:target duplex.

41. The method of claim 39, wherein said probe composition further comprises at least one helper **oligonucleotide** that facilitates formation of the probe:target duplex.

42. The method of claim 41, wherein said at least one helper **oligonucleotide** is selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3.

43. A kit for detecting the presence of Actinomycetes nucleic acids in a test sample, comprising: (a) a probe composition comprising an **oligonucleotide** probe that hybridizes under a high stringency condition to an Actinomycetes nucleic acid target region corresponding to E. coli 23S rRNA nucleotide positions 1986-2064 to form a detectable target:probe duplex, said **oligonucleotide** probe having a length of up to 100 nucleotide bases and comprising at least 25 contiguous nucleotides contained within the sequence of SEQ ID NO:10 or the complement thereof, and wherein under said hybridization condition said **oligonucleotide** probe specifically hybridizes to nucleic acids present in Corynebacterium aquaticum, Corynebacterium jeikieum, Corynebacterium xerosis, Micrococcus luteus, Propionibacterium acnes, Mycobacterium

Mycobacterium simiae, Mycobacterium avium, Mycobacterium scrofulaceum, Mycobacterium gordonae, Mycobacterium kansasii, Mycobacterium smegatis, Mycobacteriumfortuitum, Mycobacterium gastri, Mycobacterium xenopi, Mycobacterium marinum and Mycobacterium phlei; and (b) printed instructions specifying, in order of implementation, the steps to be followed for detecting said Actinomycetes nucleic acid by detecting a complex between the **oligonucleotide** probe and an Actinomycetes nucleic acid target, wherein said probe composition and said printed instructions are in packaged combination.

SUMM . . . of EDTA and EGTA. The oligonucleotide probe may be made of DNA, but also may include at least one nucleotide **analog**. For example, the probe may include at least one nucleotide **analog** that has a methoxy group at the 2' position of a ribose moiety. In one embodiment the invented oligonucleotide probe. . .

SUMM . . . probe:target duplex under high stringency hybridization conditions. At least one of the helper oligonucleotides can include at least one nucleotide **analog**. An example of a highly preferred nucleotide **analog** would be one in which a ribose moiety has a methoxy group disposed at the 2' position. In a highly. . .

SUMM . . . is 2'-deoxyribose. The sugar of a 5'-nucleotide contains a hydroxyl group (--OH) at the 5'-carbon-5 position. The term also includes **analogs** of naturally occurring nucleotides and particularly includes **analogs** having a methoxy group at the 2' position of the ribose (OMe). As used herein, methoxy oligonucleotides containing "T" residues. . .

SUMM . . . hydrogen bonding with a nucleotide, and would exclude units having as a component one of the five nucleotide bases or **analogs** thereof.

SUMM . . . replaced with a different linkage, such as a phosphorothioate linkage, a methylphosphonate linkage, or a neutral peptide linkage. Nitrogenous base **analogs** also may be components of oligonucleotides in accordance with the invention.

DETD . . . CGCCGAGTCTGTGGTTGAGACAGTGGG (SEQ ID NO:10) or the complement thereof. The oligonucleotides may be RNA and DNA equivalents, and may contain nucleotide **analogs**.

DETD . . . "helper oligonucleotides" or simply "oligonucleotides" embrace polymers of native nucleotides as well as polymers that include at least one nucleotide **analog**.

DETD Backbone-modified oligonucleotides, such as those having phosphorothioate or methylphosphonate groups, are examples of **analogs** that can be used in conjunction with oligonucleotides of the present invention. These modifications render the oligonucleotides resistant to the nucleolytic activity of certain polymerases or to nuclease enzymes. Other **analogs** that can be incorporated into the structures of the oligonucleotides disclosed herein include peptide nucleic acids, or "PNAs." The PNAs. . .

1. An **oligonucleotide** probe that specifically hybridizes to an Actinomycetes nucleic acid target region corresponding to E. coli 235 rRNA nucleotide positions 1986-2064 under a high stringency hybridization condition to form a detectable probe:target duplex, said **oligonucleotide** probe having a length of up to 100 nucleotides and comprising at least 17 contiguous nucleotides contained within the sequence. . .

2. The **oligonucleotide** probe of claim 1, wherein said probe comprises at least 25 contiguous nucleotides contained within the sequence of SEQ ID. . .

3. The **oligonucleotide** probe of claim 2, wherein said probe comprises at least 29 contiguous nucleotides contained within the sequence of SEQ ID. . .

4. The **oligonucleotide** probe of claim 1, wherein the high stringency hybridization condition is provided by 0.48 M sodium phosphate buffer, 0.1% sodium. . .

5. The **oligonucleotide** probe of claim 1, wherein the high stringency hybridization condition is provided by 0.6 M LiCl, 1% lithium lauryl

surface;. . .

6. The **oligonucleotide** probe of claim 1, wherein said **oligonucleotide** probe comprises DNA.

7. The **oligonucleotide** probe of claim 1, wherein said **oligonucleotide** probe comprises at least one nucleotide **analog**.

8. The **oligonucleotide** probe of claim 7, wherein said at least one nucleotide **analog** comprises a **methoxy** group at the 2' position of a ribose moiety.

9. The **oligonucleotide** probe of claim 1, wherein said **oligonucleotide** probe has a sequence selected from the group consisting of SEQ ID NO:1 or the complement thereof, SEQ ID NO:2. . . .

10. The **oligonucleotide** of claim 9, wherein said sequence is given by SEQ ID NO:2 or SEQ ID NO:3, said **oligonucleotide** being a helper **oligonucleotide**.

11. The **oligonucleotide** probe of claim 9, further comprising a detectable label.

12. The **oligonucleotide** probe of claim 11, wherein the detectable label is a **chemiluminescent** label or a radiolabel.

13. The **oligonucleotide** probe of claim 9, wherein said sequence is given by SEQ ID NO:1.

14. The **oligonucleotide** probe of claim 13, wherein said **oligonucleotide** probe further comprises a detectable label.

15. The **oligonucleotide** probe of claim 14, wherein the detectable label is an acridinium ester.

16. A probe composition for detecting nucleic acids of Actinomycetes bacteria, comprising: an **oligonucleotide** probe that hybridizes under a high stringency condition to an Actinomycetes nucleic acid target region corresponding to E. coli 23S rRNA nucleotide positions 1986 - 2064 to form a detectable target:probe duplex, wherein said **oligonucleotide** probe has a length of up to 100 nucleotide bases and comprises at least 17 contiguous nucleotides contained within the sequence of SEQ ID NO: 10 or the complement thereof, and wherein under said hybridization condition said **oligonucleotide** probe specifically hybridizes to nucleic acids present in Corynebacterium aquaticum, Corynebacterium jeikieum, Corynebacterium xerosis, Micrococcus luteus, Propionibacterium acnes, Mycobacterium chelonae,. . .

17. The probe composition of claim 1, wherein said **oligonucleotide** probe has a length of up to 100 nucleotide bases and comprises at least 25 contiguous nucleotides contained within the. . .

18. The probe composition of claim 16, wherein the **oligonucleotide** probe comprises DNA.

21. The probe composition of claim 16, wherein said **oligonucleotide** probe comprises the sequence of SEQ ID NO:1 or the complement thereof.

22. The probe composition of claim 16, wherein the length of said **oligonucleotide** probe is up to 60 bases.

23. The probe composition of claim 16, wherein said **oligonucleotide** probe has the length and sequence of SEQ ID NO:1.

24. The probe composition of claim 16, wherein said **oligonucleotide** probe further comprises a detectable label.

25. The probe composition of claim 22, wherein said **oligonucleotide** probe further comprises a detectable label.

probe further comprises a detectable label.

27. The probe composition of any one of claims 24, 25 or 26 wherein the detectable label is a **chemiluminescent** label or a radiolabel.

28. The probe composition of claim 27, wherein the **chemiluminescent** label is an acridinium ester.

29. The probe composition of claim 27, further comprising at least one helper **oligonucleotide** that facilitates formation of the detectable probe:target duplex under said hybridization conditions.

30. The probe composition of claim 29, wherein said at least one helper **oligonucleotide** includes at least one nucleotide **analog**.

31. The probe composition of claim 30, wherein said at least one nucleotide **analog** comprises a ribose moiety having a **methoxy** group disposed at the 2' position.

32. The probe composition of claim 29, wherein said at least one helper **oligonucleotide** has a sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3.

. . . . Actinomycetes in a test sample, comprising the steps of: (a) providing to said test sample a probe composition comprising an **oligonucleotide** probe that hybridizes under a high stringency condition to an Actinomycetes nucleic acid target region corresponding to E. coli 23S rRNA nucleotide positions 1986-2064 to form a detectable target:probe duplex, said **oligonucleotide** probe having a length of up to 100 nucleotide bases and comprising at least 25 contiguous nucleotides contained within the sequence of SEQ ID NO:10 or the complement thereof, and wherein under said hybridization condition said **oligonucleotide** probe specifically hybridizes to nucleic acids present in Corynebacterium aquaticum, Corynebacterium jeikieum, Corynebacterium xerosis, Micrococcus luteus, Propionibacterium acnes, Mycobacterium chelonae,. . .

38. The method of claim 33, wherein the **oligonucleotide** probe has the length and sequence of SEQ ID NO: 1.

39. The method of claim 38, wherein the **oligonucleotide** probe comprises a detectable label.

41. The method of claim 39, wherein said probe composition further comprises at least one helper **oligonucleotide** that facilitates formation of the probe:target duplex.

42. The method of claim 41, wherein said at least one helper **oligonucleotide** is selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3.

. . . . kit for detecting the presence of Actinomycetes nucleic acids in a test sample, comprising: (a) a probe composition comprising an **oligonucleotide** probe that hybridizes under a high stringency condition to an Actinomycetes nucleic acid target region corresponding to E. coli 23S rRNA nucleotide positions 1986-2064 to form a detectable target:probe duplex, said **oligonucleotide** probe having a length of up to 100 nucleotide bases and comprising at least 25 contiguous nucleotides contained within the sequence of SEQ ID NO:10 or the complement thereof, and wherein under said hybridization condition said **oligonucleotide** probe specifically hybridizes to nucleic acids present in Corynebacterium aquaticum, Corynebacterium jeikieum, Corynebacterium xerosis, Micrococcus luteus, Propionibacterium acnes, Mycobacterium chelonae,. . . order of implementation, the steps to be followed for detecting said Actinomycetes nucleic acid by detecting a complex between the **oligonucleotide** probe and an Actinomycetes nucleic acid target,

packaged combination.

L13 ANSWER 7 OF 8 USPATFULL on STN
1999:170726 Base-protected nucleotide **analogs** with protected thiol groups.
Hanna, Michelle M., Norman, OK, United States
The Board of Regents of the University of Oklahoma, Norman, OK, United States (U.S. corporation)
US 6008334 19991228
APPLICATION: US 1997-899022 19970723 (8)
PRIORITY: US 1996-22573P 19960724 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to protected thiol **analogs** of pyrimidine bases for syntheses of DNA and RNA by chemical or enzymatic methods. The subject **analogs** include reagents suitable for DNA or RNA synthesis via phosphoramidite, H-phosphonate or phosphotriester chemistry as well a reagents suitable for use by RNA and DNA polymerase, including thermostable polymerases employed by PCR or other nucleic acid amplification techniques. The nucleotide **analogs** synthesized by methods of this invention can thus be incorporated into oligonucleotides or polynucleotides, deprotected and derivatized with a functional group. In some cases the protecting groups are themselves antigenic and may be left on the oligonucleotides or polynucleotides for detection with antibodies. A method of synthesizing oligonucleotides with a functional group using the subject nucleotide **analogs** is also provided.
CLM What is claimed is:
1. A nucleotide **analog** of the formula: ##STR8## wherein R_1 is --H, --OH, a mono, di, or triphosphate group, or --OR_4 ; R_2 is --H, --OH, a mono, di, or triphosphate group, a phosphoramidite group, a phosphorothioamidite group, a phosphonate group, an O-substituted monophosphate group, --OR_4, or a solid support bonded via an O at the 3' position; R_3 is --H, --OH, a mono, di, or triphosphate group, or --OR_4 ; R_4 is a lower alkyl or a protecting group; and B is a modified pyrimidine base comprising a protected thiol group attached at the 5 position on said base that is not involved in Watson-Crick base pairing or does not disrupt normal Watson-Crick base pairing, said protected thiol group selected from the group consisting of a thiodinitrophenyl group, a thioalkyldinitrophenyl group, an alkyldisulfide group, and an -S-phenylacetamidomethyl group; wherein said alkyl is a lower alkyl; and said protected thiol group being stable under conditions of chemical nucleic acid synthesis and/or conditions of enyzmatic nucleic synthesis and being convertible to a reactive thiol after said synthesis.

2. The nucleotide **analog** of claim 1 wherein said base is selected from the group consisting of cytosine and uracil.

3. The nucleotide **analog** of claim 1 wherein said base compromises an additional protecting group on a reactive moiety of said base.

4. The nucleotide **analog** of claim 3 wherein said base is selected from the group consisting of N^4 -anisoyl cytosine, N^4 -benzoyl cytosine, N^4 -isobutyryl cytosine and N^4 -acetyl cytosine.

5. The nucleotide **analog** of claim 1 wherein said protected thiol group on said base is selected from the group consisting of thiodinitrophenyl (-SDNP), thioalkyldinitrophenyl (-S-R_{10} -DNP), and alkyldisulfide (-S-S-R_{10}) wherein R_{10} is a lower alkyl.

6. The nucleotide **analog** of claim 1, wherein the protecting group of R_1 is selected from the group consisting of p-(dimethoxytrityl), p-(monomethoxytrityl), fluorenylmethyloxycarbonyl, levuloyl and 9-phenylxanthene-9-yl.

7. The nucleotide **analog** of claim 1, wherein protecting group of R_3 is selected from the group consisting of 1-(2-chloro-4-methylphenyl)-4-**methoxy**-4-piperidinium, 2'-acetal, o-nitrobenzyl, tert-butyldimethyl silyl, tetrahydrofuranyl and 4-methoxytetrahydropyranyl.

8. The nucleotide **analog** of claim 1 wherein R_1 is --OR_4 wherein R_4 is a protecting group and R_2 is a phosphoramidite group.

9. The nucleotide **analog** of claim 8 wherein said base is cytosine, or uracil.

10. The nucleotide **analog** of claim 9 wherein said base comprises an additional protecting group on a reactive moiety of said base.

11. The nucleotide **analog** of claim 9 wherein said base is selected from the group consisting of N^4 -anisoyl cytosine, N^4 -benzoyl cytosine, N^4 -isobutyryl cytosine and N^4 -acetyl cytosine.

12. The nucleotide **analog** of claim 8 wherein said protected thiol group of said base is selected from the group consisting of thiodinitrophenyl (-SDNP), thioalkyldinitrophenyl (-S-R_{10} -DNP), and alkyldisulfide (-S-S-R_{10}) wherein R_{10} is a lower alkyl.

13. The nucleotide **analog** of claim 8 wherein the protecting group of R_1 is selected from the group consisting of p-(dimethoxytrityl), p-(monomethoxytrityl), fluorenylmethyloxycarbonyl, levuloyl, and 9-phenylxanthene-9-yl.

14. The nucleotide **analog** of claim 8, wherein the protecting group of R_3 is selected from the group consisting of 1-(2-chloro-4-methylphenyl)-4-**methoxy**-4-piperidinyl, 2'-acetal, o-nitrobenzyl, tert-butyldimethyl silyl, tetrahydrofuranyl and 4-methoxytetrahydropyranyl.

15. The nucleotide **analog** of claim 1 or 8, wherein said phosphoramidite of R_2 is represented by the formula: ##STR9## R_6 is a lower alkyl, cyanoethyl or a substituted lower alkyl; and R_7 and R_8 are independently lower alkyls, or when taken together with the nitrogen to which they are attached comprise one of the groups: ##STR10##

16. The nucleotide **analog** of claim 1 wherein R_1 is --OR_4 wherein R_4 is a protecting group and R_2 is a phosphorothioamidite group.

17. The nucleotide **analog** of claim 1 or 16, wherein said R_2 is a phosphorothioamidite represented by the formula: wherein R_6 is a lower alkyl, cyanoethyl or a substituted lower alkyl; and R_7 and R_8 are independently lower alkyls, or when taken together with the nitrogen to which they are attached comprise one of the groups: ##STR11##

18. The nucleotide **analog** of claim 1 wherein R_2 is an O-substituted monophosphate group selected from the group consisting of -2-chlorophenyl monophosphate, O-2,5-dichlorophenyl monophosphate, O-2,2,2-trichloroethyl monophosphate and the N oxide of 4-methoxypyridine-2-methylene monophosphate.

19. A nucleotide **analog** of the formula: wherein R_1 is --H, --OH, a mono, di, or triphosphate group, or --OR_4 ; R_2 is a

phosphoramidite group; R_3 is --H, --OH, a mono, di, or triphosphate group, or $--OR_4$; R_4 is a lower alkyl or a protecting group; and B is a modified pyrimidine base comprising a protected thiol group attached at the 5 position on said pyrimidine base, said protected thiol group selected from the group consisting of a thiodinitrophenyl group, a thioalkyldinitrophenyl group, an alkyldisulfide group, and a -S-phenylacetamidomethyl group; wherein said alkyl is a lower alkyl.

20. The nucleotide **analog** of claim 19 wherein said base is selected from the group consisting of N^4 -anisoyl cytosine, N^4 -benzoyl cytosine, N^4 -isobutyryl cytosine and N^4 -acetyl cytosine.

21. The nucleotide **analog** of claim 19, wherein R_1 is $--OR_4$ and R_4 is a protecting group selected from the group consisting of p-(dimethoxytrityl), p-(monomethoxytrityl), fluorenylmethyloxycarbonyl, levuloyl, and 9-phenylxanthene-9-yl.

22. The nucleotide **analog** of claim 19 wherein said phosphoramidite of R_2 is represented by the formula: ##STR12## wherein R_6 is a lower alkyl, cyanoethyl or substituted lower alkyl; and R_7 and R_8 are independently lower alkyls, or when taken together with the nitrogen to which they are attached form the groups: ##STR13##

23. The nucleotide **analog** of claim 22 wherein R_6 is 2-cyanoethyl, R_7 is isopropyl and R_8 is isopropyl.

24. The nucleotide **analog** of claim 23 wherein said base is uridine or deoxyuridine.

25. The nucleotide **analog** of claim 24 wherein R_1 is $--OR_4$ and R_4 is p-(dimethoxytrityl).

26. A nucleotide **analog** of the formula: wherein: R_1 is --H, --OH, a mono, di, or triphosphate group, or $--OR_4$; R_2 is --H, --OH, a mono, di, or triphosphate group, a phosphoramidite group, a phosphorothioamidite group, a phosphonate group, an O-substituted monophosphate group, $--OR_4$, or a solid support bonded via an O at the 3' position; R_3 is --H, --OH, a mono, di, or triphosphate group, or $--OR_4$; R_4 is a lower alkyl or a protecting group; and B is a modified pyrimidine base comprising a protected thiol group attached at the 5 position on said base, said protected thiol group selected from the group consisting of a thiodinitrophenyl group, a thioalkyldinitrophenyl group, an alkyldisulfide group, and a -S-phenylacetamidomethyl group; wherein said alkyl is a lower alkyl.

27. The nucleotide **analog** of claim 26 wherein said base is cytosine, or uracil.

28. The nucleotide **analog** of claim 26 wherein said protected thiol group on said base is selected from the group consisting of thiodinitrophenyl (-SDNP), thioalkyldinitrophenyl ($-S-R_{10}$ -DNP), S-phenylacetamidomethyl($-S-CH_2$ $NHCOCH_2$ Ph) wherein R_{10} is a lower alkyl.

29. The nucleotide **analog** of claim 26, wherein R_1 is a monophosphate, a diphosphate, or a triphosphate.

30. An **oligonucleotide** containing the incorporated form of the nucleotide **analog** of any one of claims 1, 8, 16, 19, or 26.

31. A method of producing the nucleotide **analog** of any one of claims 1, 8, 16, 19, or 26 which comprises preparing the thiol-protected nucleoside or nucleotide base wherein said thiol is attached to the 5

position on said base, reacting said nucleoside or nucleotide base under conditions to effect conversion of said base to a phosphoramidite, phosphorothioamidite, phosphonate, O-substituted monophosphate or phosphate nucleotide **analog** and under conditions which do not destroy the protected thiol; and recovering said **analog**.

32. A method of synthesizing a nucleic acid having an attached functional group which comprises incorporating a thiol-protected nucleotide **analog** of any one of claims 1, 8, 16, 19, or 26 into a nucleic acid by a chemical or enzymatic method of nuicleic acid synthesis; recovering said nucleic acid containing said **analog;** deprotecting the **analog** of said nucleic acid to produce a nucleic acid containing a reactive thiol group; treating the reactive thiol group with a thiol modifying reagent to thereby attach a functional group and produce said nucleic acid with an attached functional group; and recovering said nucleic acid with said attached functional group.

33. The method of claim 32 wherein said functional group is selected from the group consisting of a photocrosslinker, a crosslinker, a reporter molecule, a radioisotope, a fluorescent group, a spin label, **chemiluminescent** or an antigenic group.

34. The method of claim 33 wherein said photocrosslinker is an aryl azide.

35. The method of claim 33 wherein said reporter group is biotin, an enzyme, or a fluorescent molecule.

36. The method of claim 33 wherein said fluorescent group is fluorescein.

37. A method for producing a deoxyuridine **analog** having a protected thiol group on the 5 position, comprising: reacting a diacetyl 5-thiocyanate deoxyuridine to form a 5-dinitrophenylthio deoxyuridine; converting said 5-dinitrophenylthio deoxyuridine to a phosphoramidite, phosphorothioamidite, phosphonate, O-substituted monophosphate or phosphate nucleotide **analog** under conditions which do not destroy the 5-dinitrophenylthio group; and recovering said 5-dinitrophenylthio deoxyuridine **analog**.

TI Base-protected nucleotide **analogs** with protected thiol groups

AB The present invention is directed to protected thiol **analogs** of pyrimidine bases for syntheses of DNA and RNA by chemical or enzymatic methods. The subject **analogs** include reagents suitable for DNA or RNA synthesis via phosphoramidite, H-phosphonate or phosphotriester chemistry as well a reagents suitable for. . . use by RNA and DNA polymerase, including thermostable polymerases employed by PCR or other nucleic acid amplification techniques. The nucleotide **analogs** synthesized by methods of this invention can thus be incorporated into oligonucleotides or polynucleotides, deprotected and derivatized with a functional. . . oligonucleotides or polynucleotides for detection with antibodies. A method of synthesizing oligonucleotides with a functional group using the subject nucleotide **analogs** is also provided.

SUMM The present invention is directed to thiol-protected pyrimidine nucleotide **analogs** which can be used, as one example, for syntheses of DNA and RNA by chemical or enzymatic methods. The subject **analogs** include reagents suitable for DNA or RNA synthesis via phosphoramidite, H-phosphonate or phosphotriester chemistry as well as reagents suitable for. . . and DNA polymerases, including thermostable polymerases employed by PCR or other nucleic acid amplification techniques. Methods of synthesizing the nucleotide **analogs** are also provided by the present invention. The nucleotide **analogs** of this invention can thus be incorporated into oligonucleotides or polynucleotides, deprotected, and then derivatized with a functional group.

SUMM . . . reporter groups, allowing site-specific delivery of therapeutics, and introducing crosslinkers. Such modifications can occur

linkages, modified sugars or modified bases. Additionally, 5'- or 3'-end conjugates of the oligonucleotides represent another class of modified oligonucleotides. The present invention relates to base-modified nucleotide **analogs** with protected thiol groups; these **analogs** are intermediates for chemical or enzymatic synthesis of oligonucleotides and polynucleotides.

SUMM . . . other potential uses, such as the site-directed delivery of therapeutics, utility as anti-sense therapeutics, and utility as diagnostic probes. Nucleotide **analogs** can be introduced into nucleic acids either enzymatically, utilizing DNA and RNA polymerases, or chemically, utilizing manual or automated synthesis.. . . of such oligonucleotides by automated synthesis utilizing, for example, phosphoramidite nucleotides allows for incorporation of a broad range of nucleotide **analogs** without the restraints for specific substrate conformation of the nucleotides that is imposed by most polymerases. Often, nucleotide **analogs** containing photoreactive crosslinking groups are introduced into oligonucleotides to probe protein-nucleic acid interactions via photocrosslinking (for a partial review, see. . . modified with a thiol modifying reagent the normal Watson-Crick base of the nucleotide is drastically affected, making this and similar **analogs** generally unsuitable for use in enzymatic nucleic acid synthesis.

SUMM Other **analogs** involving modifications at the C5 position of deoxyuridine have also been previously reported. The thiol-containing **analog**, 5-thiocyanatodeoxyuridine phosphoramidite, provided a 5-mercaptodeoxyuridine moiety within the oligonucleotide following reduction of the thiocyanate (Bradley & Hanna, 1992). However, the. . . variable stability during synthesis of both the nucleotide and the oligonucleotide and therefore this compound did not represent an ideal **analog** for incorporation of 5-thiol modified nucleotides into nucleic acids. The syntheses of a series of phosphoramidites containing alkylthiol tethers at the C5 position of deoxyuridine has been reported (Goodwin & Glick, 1993). The thiol groups in these **analogs** are attached to the ring by either a three, four, or five carbon chain. The presence of the carbon chains. . . makes the minimal distance between the molecular probes and the oligonucleotide greater than that which can be achieved with our **analog**. In addition, these compounds represent alkyl thiol **analogs** which have a lower reactivity for modification of the thiol group than the 5-mercaptopyrimidine **analog**. This is due to an increase in acidity of 5-mercaptopyrimidines (pKa.about.5-5.6) over alkylthiol moieties (pKa.about.8-10). Phosphoramidites containing alkylthiol tethers at. . . have also been prepared, but the position of this modification results in a disruption of the Watson-Crick base pairing. These **analogs** have been used mainly for preparing disulfide cross-links in DNA for studies involving stem loop and triple helical structures (Glick,. . .

SUMM . . . protected carboxylic acids and alkyl amines have also been described (Bergstrom et al., 1991). The protected functional groups in these **analogs** are not attached directly to the ring but are positioned at the end of carbon chains. These groups are not. . . Broom, 1991; Waters & Connolly, 1992; Xu et al., 1992b) and 6-thioinosine (Clivio et al., 1992a) have been reported. These **analogs** can occupy internal positions within an oligonucleotide and can serve as photochemical crosslinkers. However, they cannot be further modified without disrupting Watson-Crick base pairing, and therefore, as photocrosslinking probes, these **analogs** are only useful for evaluating interactions which occur directly with the nucleotide base. Modification with other molecular probes (e.g., fluorescent. . . pairing. In addition, the deprotection of oligonucleotides containing these thiol-modified nucleotides must be carefully monitored to prevent conversion of these **analogs** to the corresponding oxygen and nitrogen derivatives.

SUMM Described herein are nucleotide **analogs** which can be used for site-specific modification of DNA or RNA at internal and terminal positions within the DNA or. . .

SUMM The present invention provides novel base protected nucleotide **analogs**, both ribonucleotides and deoxynucleotides, that contain masked thiol groups on the 5 position of pyrimidines, which is not involved in Watson-Crick base pairing. These **analogs** can be incorporated into oligonucleotides via automated synthesis and isolated with the thiol protecting group intact. After removal of the. . . by utilizing thiol-modifying reagents. This feature adds a level of specificity to the oligonucleotide modifications not present with the amino-tagged **analogs** previously described (Gibson et al, 1987), and enables examination of molecular interactions that are not directly at the nucleotide base by allowing functional groups to be placed at varying distances from the base or helix strand. Since these **analogs** have the functional group attached via the sulfur atom, some have the further advantage of being cleavable under conditions which. . .

SUMM This invention relates to pyrimidine nucleotide **analogs** which contain modified bases with protected thiol groups attached at a position on the base, preferably the 5 position, which is not involved in Watson-Crick base pairing. These nucleotide **analogs** are intermediates in chemical or enzymatic synthesis of DNA or RNA oligonucleotides and are therefore stable under conditions required for synthesis of these molecules. After synthesis, the protecting group on the **analog** is removable to generate a reactive thiol group. Once generated, the thiol group can be treated with thiol modifying reagents. . .

SUMM In particular, the nucleotide **analogs** of the present invention have the formula: ##STR1## wherein R_1 is --H, --OH, a mono, di, or triphosphate group, or. . .

SUMM Preferred nucleotide **analogs** of the present invention are the protected phosphoramidites or 5' mono, di and triphosphates of modified cytosine or uridine bases. . .

SUMM In addition, the nucleotide **analogs** of the present invention include other nucleoside phosphates, containing 3', 5', or 3',5' monophosphates, diphosphates, or triphosphates and further optionally. . .

SUMM Another aspect of this invention provides nucleic acids and oligonucleotides containing the subject nucleotide **analogs** having a protected thiol group on a base moiety of that nucleic acid or oligonucleotide. A method is also provided. . .

SUMM Yet another aspect of this invention is directed to a method of synthesizing the subject base-protected nucleotide **analogs**.

DRWD . . . at 9.8 min. (b) DNP-labeled oligonucleotide B, GTA T*GT A, eluted at 11.6 min, where T represents incorporation of the DNP-**analog** described in FIG. 1.

DRWD . . . complex a deoxynucleoside phosphoramidite was incorporated site-specifically into a single-stranded DNA oliqonucleotide via automated synthesis. In these studies, the nucleotide **analog** was modified with a masked reactive thiol group on the non-basepairing 5 position of deoxyuridine (5-S-DNP-dU, FIG. 1). The oligonucleotide was then radioactively labeled uniquely on the 5' phosphate of the nucleotide **analog** with polynucleotide kinase and [γ-^{32} P] ATP (Step 1). The radioactively labeled, base-modified oligonucleotide was then annealed to a single-stranded. . .

DETD The present invention relates to a series of novel nucleotide **analogs** which are masked synthons for use as intermediates in chemical or enzymatic synthesis of nucleic acids, including synthesis of both oligonucleotides and polynucleotides. The nucleotide **analogs** of this invention, which contain a protected thiol group, can thus be incorporated into DNA or RNA under standard synthetic. . . thiol protecting group. This stability of the thiol protecting group permits site-selective introduction into a nucleic acid of the nucleotide **analog** in a manner which facilitates later addition of a functional group at that site. Thus, the subject oligonucleotides (or polynucleotides) can contain one (or more) of the subject nucleotide **analogs**.

DETD In particular, the nucleotide **analogs** of the present invention are intermediates for the chemical synthesis of DNA and RNA by manual or automated techniques and. . .

DETD . . . removable under conditions which do not disrupt the integrity

nucleotide **analog** of the present invention has been incorporated into an oligonucleotide, for example, the protected thiol group can be converted to. . .

DETD The bases of this invention also include any related base **analog** that is capable of base pairing with a guanine or adenine, the corresponding protected **analogs** as set forth above for use in chemical synthetic methods to produce DNA and RNA. For example, such base **analogs** include, but are not limited to, pseudocytosine, isopseudocytosine, 4-acetylcytosine, 2'-O-methylcytosine, dihydrouracil, 2'-O-methyluracil, 2'-O-methyl-pseudouracil, 1-methylpseudouracil, 3-methylcytosine. Bases attached to a ribose. . .

DETD The nucleotide **analogs** of the present invention can be prepared by adding a protected thiol group to the base moiety of the desired nucleoside. The so-modified nucleoside can then be phosphorylated to produce a nucleotide **analog** phosphate compound of this invention using conventional phosphorylation techniques. To produce a phosphoramidite, phosphonate, phosphorothioamidite or O-substituted monophosphate of this. . . by addition of the desired protecting group by standard methodology. This protection step(s) is (are) followed by conversion to the nucleotide-**analog** phosphoramidite, phosphorothioamidite, phosphonate or O-substituted monophosphate by reaction of the 3'OH of the nucleoside with the appropriate modifying group. Similarly,. . .

DETD . . . and U nucleosides can then be reduced to 5-mercapto nucleosides for further derivatization as described herein to produce the nucleotide **analogs** of the present invention.

DETD . . . 5'-O-(4,4'-dimethoxytrityl)-5-S-(2,4-dinitrophenyl) mercapto-2'-deoxyuridine-3'-O-(2-cyanoethyl-N,N'-diisopropyl) phosphoramidite is more reproducible and the product is more stable than 5-thiocyanatodeoxyuridine phosphoramidite and represents a more desirable **analog**. A variety of functional groups can be attached to the reactive thiol. Once this modified nucleotide is incorporated into an. . . making it available for further derivitization with thiol-specific agents. This method has the advantage that an oligonucleotide tagged with this **analog** can be synthesized and stored for long periods without removal of the protecting group. When needed, an aliquot can then. . .

DETD One method used to produce one of the nucleotide **analog** phosphoramidites of this invention and described in detail in Example 1 below is shown in FIG. 1. Briefly, as depicted,. . .

DETD . . . the exocyclic amine groups on the base moiety may be necessary before the final reaction step which produces the nucleotide **analogs** of this invention.

DETD . . . also provide information and protocols to attach nucleotides to solid supports which protocols are useful for attaching the base-protected nucleotide **analogs** of this invention.

DETD . . . has been described herein above. All of these reaction schemes can be used to produce the corresponding thiol protected cytosine **analogs**. If necessary, various protecting groups for the 5'OH, 3'OH or 2'OH groups as well as the exocyclic amines can be. . .

DETD Another aspect of this invention relates to the oligonucleotides or polynucleotides containing the nucleotide **analogs** of this invention and a method of preparing such nucleic acids using the subject nucleotide **analogs**. oligonucleotides and polynucleotides of this invention are made by standard methods of chemical (automated or manual) synthesis or enzymatic synthesis of DNA and RNA. Such methods are well known in the art. In chemical synthesis, the nucleotide **analog** of this invention is substituted for a particular nucleotide at the desired point in the synthesis.

DETD After incorporation of the nucleotide **analog** and complete synthesis of the oligonucleotide or polynucleotide, the thiol can be deprotected and reacted with any number of thiol-modifying. . . be accomplished by treatment with β-mercaptoethanol or by other means of reducing sulfides. In a preferred method for the DNP **analog**, deprotection is accomplished by treating the oligonucleotide with 1.4M BME at 45° C. for 4 hours or at room temperature. . .

DETD . . . another aspect of this invention provides a method of preparing the nucleotide **analogs** of the invention. In particular, this method involves preparing a thiol protected nucleoside or nucleotide base wherein said thiol is. . . nucleotide base under conditions to effect conversion of said base to a phosphoramidite, phosphorothioamidite, phosphonate, O-substituted monophosphate or phosphate nucleotide **analog** and under conditions which do not destroy the protected thiol; and recovering said **analog**. In accordance with this invention, this method is accomplished as described above for synthesis of the subject nucleotide **analogs**. Recovery of the **analogs** can be accomplished by HPLC, FPLC or other chromatographic separation techniques.

DETD . . . this invention provides a method of synthesizing a nucleic acid with a functional group by incorporating a thiol protected nucleotide **analog** in accordance with this invention into a nucleic acid by a chemical or enzymatic method for nucleic acid synthesis; recovering the nucleic acid containing the **analog**; deprotecting the **analog** of that nucleic acid to produce a nucleic acid containing a reactive thiol group; reacting the reactive thiol group with. . . 5 to about 100 nucleotides. Polynucleotides range in size from 100 nucleotides to 10 kb or more. Recovery of the **analogs** can be accomplished by HPLC, FPLC, other chromatographic techniques, extraction, phase separation or precipitation.

DETD . . . 7.76) 260, 334 nm. (The broad melting point range may indicate the presence of 5,5"-dithiobis(3',5'-diacetyldeoxyuridine). On the average, 7% disulfide **analog** was present in the sample as determined by UV absorbance (λmax 335 nm) in the presence and absence of DTT. . .

DETD . . . A series of three modified oligonucleotides were prepared on a 40 nmole scale using a Trityl On method. The thiol-protected **analog**, Compound 7, was manually dissolved into anhydrous acetonitrile at a concentration of 0.027 M and the solution was attached to. . . lower than the concentration of the commercial phosphoramidites used in these syntheses (0.05 M), the coupling time for the modified **analog** was increased by 120 seconds. Syntheses of the corresponding unmodified oligonucleotides were also performed. The sequences of the oligonucleotides are. . .

DETD . . . 7) containing a protected thiol group at the 5 position of the mercaptopyrimidine ring is described (FIG. 1). This DNP-labeled **analog** was site-specifically incorporated internally into a series of oligonucleotides. Significant differences in overall synthetic yields were not observed between oligonucleotides made with normal deoxynucleoside phosphoramidites and those which contained the **analog**, indicating that the modified phosphoramidite is a suitable reagent for automated oligonucleotide synthesis. Enzymatic digestion of the DNP-labeled oligonucleotide established. . .

DETD Synthesis of the deoxyuridine phosphoramidite **analog** (Compound 7) was carried out following the sequence of reactions outlined in FIG. 1. Compounds 2-7 have not been previously. . .

DETD . . . of the thiocyanato group. Therefore, a synthetic approach was developed which required reduction of the thiocyanato compound to the mercapto **analog** prior to removal of the acetyl groups (FIG. 1). Thibcyanation of the 3',5'-O-diacetyl-2'-deoxyuridine (1) was achieved following the literature procedure. . .

DETD Reduction of the thiocyanato derivative 2 to the mercapto **analog** (Compound 3) was achieved by reaction with DTT in a solvent mixture of MeOH and 0.1 M EDTA (pH=7.8). This reaction is a variation of a reported procedure (Lin et al., 1988) for the reduction of 3'-azido-2',3'-dideoxy-5-thiocyanatouridine; this mercapto **analog** however was not isolated. Purification of Compound 3 was achieved by acidification of the reaction mixture followed by concentration of. . .

DETD . . . a fume hood. This synthesis of Compound 3 was designed for the preparation of both 5-alkyldithio and 5-alkylthio or 5-arylthiopyrimidine **analogs**. To prevent degradation of the 5-alkyldithio compounds by DTT, purification of Compound 3 was necessary. This is not the case. . .

DETD . . . These studies indicated the DNP group was stable during

2,4-DNP **analog** 4 was achieved by reaction with 2,4-dinitrofluorobenzene in anhydrous CH_3 CN with Et_3 N present as a catalyst (Xu et. . . . solvents, which aided in the purification of the material. More importantly, protection of the sulfur inhibits the oxidation of the **analog** to the disulfide compound, a potential reaction of 5-mercaptopyrimidines (Kalman & Bardos, 1967).

DETD . . . 5-arylthiol ethers of deoxyuridine has been previously described. Bergstrom et al. has prepared a series of alkyl and aryl mercaptodeoxyuridine **analogs** using a palladium-mediated reaction between 5-(chloromercuri)-2'-deoxyuridine and the appropriate disulfide (Bergstrom et al., 1991). Although this method offers a simple route to arylthiol ether **analogs** it was not selected for study. Development of a more versatile intermediate such as Compound 3 which can be easily converted into various types of **analogs** including disulfides was desired for future studies. Additionally, the synthetic utility of the method has not been fully evaluated and therefore formation of the desired dinitrophenyl **analog** (Compound 5) was uncertain.

DETD . . . dependent on the reaction conditions (Greene, 1981). Successful removal of the acetyl groups was achieved by reaction of the diacetyl **analog** (Compound 4) with NaOMe in MeOH without an apparent loss or modification of the DNP group as judged by NMR. . . .

DETD . . . was not successful (unpublished results), this reaction was not attempted for the synthesis of Compound 6. Conversion to the 5'-dimethoxytrityl **analog** (Compound 6) was achieved by reaction of Compound 5 with DMT+ BF_4 -- in the presence of DBMP in CH_3. . . .

DETD . . . yielding reaction in this synthetic sequence. However, approximately 20% of the starting material, Compound 5, was converted into the 3',5'-bis(dimethoxytrityl) **analog**, which was isolated and converted back into 4 for recycling into the synthesis.

DETD Internal incorporation of the phosphoramidite **analog**, Compound 7, into series of oligonucleotides has been accomplished using an automated DNA synthesizer. The natural oligonucleotides with corresponding sequences. . .

DETD . . . #13, 1987). However, studies involving reaction of the DNP-labeled nucleoside 5 with conc NH_4 OH at 55° C. indicated the **analog** was not stable to these conditions but was stable at room temperature (data not shown). As successful removal of standard. . .

DETD . . . at 330 nm is associated with the presence of either the dinitrophenylthiol ether or the 5-mercaptopyrimidine moiety. If the DNP-labeled **analog** had undergone modification during automated DNA synthesis, deprotection, or purification, extraneous peaks were predicted to appear in this chromatogram.

DETD . . . is consistent with our observations that a 20-mer RNA containing 5-SH-UTP co-migrates in this gel system with 20-mer containing no **analog** (He et al., 1995). In contrast, a substantial difference is observed following modification with fluorescein, consistent with other reports of. . .

DETD . . . site in the nucleic acid, and degradation of some proteins during irradiation. A second approach involves the use of nucleotide **analogs** modified with photoreactive crosslinking groups (reviewed in M. Hanna, 1989, Methods in Enzymology). These groups are chemically inert in the. . .

DETD For such mechanistic studies a variety of nucleotide **analogs** have been developed and utilized (M. Hanna 1989, Methods in Enzymology; Bradley and Hanna, 1992). These **analogs** are either tagged with a photoreactive crosslinking group or contain functional groups that can be tagged with crosslinkers after incorporation into DNA or RNA. These **analogs** can be incorporated into nucleic acids, either enzymatically or chemically, to analyze molecular interactions in protein-nucleic acid complexes. Upon photoactivation, **analog**-tagged nuecleic acids become covalently attached to adjacent macromolecules (protein, DNA, RNA) with which they have direct interactions. Therefore, nucleic acid. . .

DETD . . . No. 2, was synthesized on an Applied Biosystems 392 DNA Synthesizer using the standard β-cyanoethyl-protected phosphoramidite method by attaching the **analog** to the fifth substrate port. After synthesis, removal of the oligonucleotide from the column, and removal of the exocyclic amine. . .

DETD To evaluate the effect of incorporation of one or more 5-SDNP-dU **analogs** into oligodeoxynucleotides (ODNs) on their hybridization properties, the ODNs shown in Table 2 were synthesized. The observed melting temperatures (T_m) showed that incorporation of one DNP **analog** decreased the melting temperature by only 1.8° C. (hybrid V), compared to the normal DNA hybrid (I), and substitution with two DNP **analogs** caused a decrease of only 4.4° C. (hybrid VI). Therefore, the effect of a single substitution is less than that. . . by a single mismatch involving unmodified nucleotides (hybrids II, III and IV vs I), and the effect of two modified **analogs** is still less than that of a single T-C mismatch (hybrid IV). Mismatches of 2 or more are commonly used for site-directed mutagenesis, which involves hybridization of the oligonucleotides to single-stranded DNA. Substitution with the DNP **analog** should therefore work as well, if not better. The use of ODNs containing one or more 5-SDNP-dU **analog** in assays requiring specific hybridization to a complementary strand is therefore quite feasible.

DETD . . . nucleobase substitutions (Jadhav et al. 1997). A more serious effect on the hybridization properties was observed for another previously described **analog**, 2-thiodeoxyuracil. Placement of even a single 2-thiodeoxyuracil **analog** into oligodeoxynucleotides (14-mers) caused a decrease in T_m of 3-4° C. (Kuimelis and Nambiar, 1994).

DETD The **analog**-modified oligonucleotide (100 pmol) was radiolabeled with T4 polynucleotide kinase using [γ^{32} P] ATP (FIG. 5, Step 1), isolated by ethanol. . .

DETD The disadvantages of RNase protection are overcome by the envisioned NAP Nuclease Protection Assays (NAP-protection assays) employing the derivatizable nucleotide **analogs** herein. The NAPs can be synthesized in large quantities, carefully evaluated (quality control assays), and then stored for future use.. . .

DETD Use of oligonucleotides or NAP probes which contain modified nucleotide **analogs** has been previously reported (21-23, 26-29). The chemical approach to preparation of site-specifically modified DNA involves incorporation of a modified. . . groups such as amines (Nagamachi, et al. 1974), carboxylic acids, thiols (Goodwin and Glick, 1993) and thiocarbonyls. Once incorporated the **analogs** are deprotected and modified post-synthetically. Convertible nucleoside phosphoramidites, mononomers containing leaving groups, have also been used to incorporate crosslinking and. . . two ends of a nucleic acid (5' or 3'). Some provide reagents which allow the incorporation of more than one **analog** at internal positions in the NAP, to allow an increase in the amount of signal produced by the NAP, there. . .

DETD Preferred versions of the nucleotide **analogs** contemplated herein have the following characteristics which make them preferable to other currently available nucleotide **analogs** for this assay:

DETD . . . therefore reaction of the probe modified oligonucleotides or NAPs with thiol-reactive alkylating agents gives virtually thiol-specific modification of the NAP. **Analogs** with reactive amino groups, which are modified by alkylating agents targeting amines, are less specifically modified. This is due to. . .

DETD . . . region close to the nucleic acid backbone. This is advantageous because the flexibility of the linker are allows the nucleotide **analogs** to adopt to different enzyme active sites (in DNA and RNA polymerases), making them generally good substrates for enzymatic incorporation. . .

DETD 5. As assessed by several functional assays, **analogs** modified through the aryl thio group of 5-S-U derivatives do not disturb normal RNA secondary or tertiary structures.

DETD . . . many fluorescent, colormetric, chemiluminescent, or antigenic reporter groups. One such antigenic reporter group is the dinitrophenyl

The assay is the same as the current method previously described, but the protected. . .

DETD 1) DNA NAPs Prepared with **Analog**-Tagged Oligonucleotide Primers

DETD Already developed for use in such a DNA NAP-protection assay is the **analog** 5-DNP-dU phosphoramidite. This **analog** can be incorporated chemically into ssDNA oligonucleotides utilizing automated synthesis. Therefore oligonucleotide primers for the PCR reaction can be made which contain multiple **analogs**, with nearly every "T" in the oligonucleotide synthesis and isolation, and we have shown that it is also retained during. . .

DETD 2) DNA NAPs Prepared with **Analog**-Tagged Deoxynucleoside Triphosphates

DETD Cosstick, R. and Douglas, M. E. (1991) Synthesis of a Dinucleoside Monophosphate **Analogue** Containing 6-N-(2-Aminoethyl)-2'-Deoxyadenosine. A Novel Approach to Sequence Specific Crosslinking in Synthetic Oligonucleotides J. Chem. Soc. Perkin Trans. 1 1035-1040.

DETD . . . Dissinger, S., Williams, B. D. and Colston, J. E. (1989) Synthesis and Characterization of 5-[4-(Azidophenacyl)thio]uridine 5'-Triphosphate, a Cleavable Photo-Cross-Linking Nucleotide **Analogue** Biochemistry 28, 5814-5820.

1. A nucleotide **analog** of the formula: ##STR8## wherein R_1 is --H, --OH, a mono, di, or triphosphate group, or --OR_4 ; R_2 is. . .

2. The nucleotide **analog** of claim 1 wherein said base is selected from the group consisting of cytosine and uracil.

3. The nucleotide **analog** of claim 1 wherein said base compromises an additional protecting group on a reactive moiety of said base.

4. The nucleotide **analog** of claim 3 wherein said base is selected from the group consisting of N^4 -anisoyl cytosine, N^4 -benzoyl cytosine, N^4. . .

5. The nucleotide **analog** of claim 1 wherein said protected thiol group on said base is selected from the group consisting of thiodinitrophenyl (-SDNP),. . .

6. The nucleotide **analog** of claim 1, wherein the protecting group of R_1 is selected from the group consisting of p-(dimethoxytrityl), p-(monomethoxytrityl), fluorenylmethyloxycarbonyl, levuloyl. . .

7. The nucleotide **analog** of claim 1, wherein protecting group of R_3 is selected from the group consisting of 1-(2-chloro-4-methylphenyl)-4-**methoxy**-4-piperidinium, 2'-acetal, o-nitrobenzyl, tert-butyldimethyl silyl, tetrahydrofuranyl and 4-methoxytetrahydropyranyl.

8. The nucleotide **analog** of claim 1 wherein R_1 is --OR_4 wherein R_4 is a protecting group and R_2 is a phosphoramidite group.

9. The nucleotide **analog** of claim 8 wherein said base is cytosine, or uracil.

10. The nucleotide **analog** of claim 9 wherein said base comprises an additional protecting group on a reactive moiety of said base.

11. The nucleotide **analog** of claim 9 wherein said base is selected from the group consisting of N^4 -anisoyl cytosine, N^4 -benzoyl cytosine, N^4. . .

12. The nucleotide **analog** of claim 8 wherein said protected thiol group of said base is selected from the group consisting of thiodinitrophenyl (-SDNP),. . .

13. The nucleotide **analog** of claim 8 wherein the protecting group of R_1 is selected from the group consisting of p-(dimethoxytrityl), p-(monomethoxytrityl), fluorenylmethyloxycarbonyl, levuloyl,. . .

14. The nucleotide **analog** of claim 8, wherein the protecting group of

R_3 is selected from the group consisting of 1-(2-chloro-4-
methylphenyl)-4-**methoxy**-4-piperidinyl, 2'-acetal, o-nitrobenzyl, tert-butyldimethyl silyl, tetrahydrofuranyl and 4-methoxytetrahydropyranyl.

15. The nucleotide **analog** of claim 1 or 8, wherein said phosphoramidite of R_2 is represented by the formula: ##STR9## R_6 is a lower. . .

16. The nucleotide **analog** of claim 1 wherein R_1 is --OR_4 wherein R_4 is a protecting group and R_2 is a phosphorothioamidite group.

17. The nucleotide **analog** of claim 1 or 16, wherein said R_2 is a phosphorothioamidite represented by the formula: wherein R_6 is a lower. . .

18. The nucleotide **analog** of claim 1 wherein R_2 is an O-substituted monophosphate group selected from the group consisting of -2-chlorophenyl monophosphate, O-2,5-dichlorophenyl monophosphate,. . .

19. A nucleotide **analog** of the formula: wherein R_1 is --H, --OH, a mono, di, or triphosphate group, or --OR_4 ; R_2 is a. . .

20. The nucleotide **analog** of claim 19 wherein said base is selected from the group consisting of N^4 -anisoyl cytosine, N^4 -benzoyl cytosine, N^4. . .

21. The nucleotide **analog** of claim 19, wherein R_1 is --OR_4 and R_4 is a protecting group selected from the group consisting of p-(dimethoxytrityl),. . .

22. The nucleotide **analog** of claim 19 wherein said phosphoramidite of R_2 is represented by the formula: ##STR12## wherein R_6 is a lower alkyl,. . .

23. The nucleotide **analog** of claim 22 wherein R_6 is 2-cyanoethyl, R_7 is isopropyl and R_8 is isopropyl.

24. The nucleotide **analog** of claim 23 wherein said base is uridine or deoxyuridine.

25. The nucleotide **analog** of claim 24 wherein R_1 is --OR_4 and R_4 is p-(dimethoxytrityl).

26. A nucleotide **analog** of the formula: wherein: R_1 is --H, --OH, a mono, di, or triphosphate group, or --OR_4 ; R_2 is --H,. . .

27. The nucleotide **analog** of claim 26 wherein said base is cytosine, or uracil.

28. The nucleotide **analog** of claim 26 wherein said protected thiol group on said base is selected from the group consisting of thiodinitrophenyl (-SDNP),. . .

29. The nucleotide **analog** of claim 26, wherein R_1 is a monophosphate, a diphosphate, or a triphosphate.

30. An **oligonucleotide** containing the incorporated form of the nucleotide **analog** of any one of claims 1, 8, 16, 19, or 26.

31. A method of producing the nucleotide **analog** of any one of claims 1, 8, 16, 19, or 26 which comprises preparing the thiol-protected nucleoside or nucleotide base. . . nucleotide base under conditions to effect conversion of said base to a phosphoramidite, phosphorothioamidite, phosphonate, O-substituted monophosphate or phosphate nucleotide **analog** and under conditions which do not destroy the protected thiol; and recovering said **analog**.

functional group which comprises incorporating a thiol-protected nucleotide **analog** of any one of claims 1, 8, 16, 19, or 26 into a nucleic acid by a chemical or enzymatic method of nuicleic acid synthesis; recovering said nucleic acid containing said **analog**; deprotecting the **analog** of said nucleic acid to produce a nucleic acid containing a reactive thiol group; treating the reactive thiol group with. . .

. . . from the group consisting of a photocrosslinker, a crosslinker, a reporter molecule, a radioisotope, a fluorescent group, a spin label, **chemiluminescent** or an antigenic group.

37. A method for producing a deoxyuridine **analog** having a protected thiol group on the 5 position, comprising: reacting a diacetyl 5-thiocyanate deoxyuridine to form a 5-dinitrophenylthio deoxyuridine; converting said 5-dinitrophenylthio deoxyuridine to a phosphoramidite, phosphorothioamidite, phosphonate, O-substituted monophosphate or phosphate nucleotide **analog** under conditions which do not destroy the 5-dinitrophenylthio group; and recovering said 5-dinitrophenylthio deoxyuridine **analog**.

L13 ANSWER 8 OF 8 USPATFULL on STN

97:15952 Assays using chemiluminescent, enzymatically cleavable substituted 1,2-dioxetanes and kits therefor.

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US 5605795 19970225

APPLICATION: US 1994-255795 19940607 (8)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Dioxetane compounds reactable with an enzyme to release optically detectable eneregy are disclosed. These compounds have the formula: ##STR1## wherein T is a cycloalkyl group or a fused polycyclo-alkylidene group bonded to the dioxetane ring through a spiro linkage; Y is a fluorescent chromophore capable of absorbing energy to form an excited energy state from which it emits optically detectable energy to return to its original energy state; X is hydrogen or an alkyl, aryl, aralkyl, alkaryl, heteroalkyl, heteroaryl, cycloalkyl or cycloheteroalkyl group, or an enzyme-cleavable group containing a bond cleavable by an enzyme to yield an electron-rich moiety bonded to the dioxetane ring, Z is hydrogen, hydroxyl or an enzyme-cleavable group containing a bond cleavable by an enzyme to yield an electron-rich moiety bonded to the dioxetane ring, at least one of X and Z being an enzyme-cleavable group, and T also includes a substituent which enhances the solubility of the dioxetane in aqueous solution, or a substituent which facilitates bonding of the dioxetane to a membrane, film, bead, polymer or polymerizable group, or a substituent which enhances the kinetics of the dioxetane enzyme degradation.

CLM What is claimed is:

1. A method of detecting an enzyme in a sample comprising the steps of: (a) providing an enzymatically cleavable **chemiluminescent** 1,2-dioxetane compound capable of producing light energy when decomposed, substantially stable at room temperature before a bond by which an enzymatically cleavable labile substituent thereof is intentionally cleaved, represented by the formula: ##STR31## wherein T is a cycloalkyl group or a fused polycyclo-alkylidene group bonded to the dioxetane ring through a spiro linkage; Y is a fluorescent chromophore capable of absorbing energy to form an excited energy state from which it emits optically detectable energy to return to its original energy state; X is hydrogen or an alkyl, aryl, aralkyl, alkaryl, heteroalkyl, heteroaryl, cycloalkyl or cycloheteroalkyl group, or an enzyme-cleavable group containing a bond cleavable by an enzyme to yield an electron-rich moiety bonded to the dioxetane ring, Z is hydrogen, hydroxyl or an enzyme-cleavable group containing a bond cleavable by an enzyme to yield an electron-rich moiety bonded to the

dioxetane ring, at least one of X and Z being an enzyme cleavable group, and T also includes a substituent which enhances the solubility of the dioxetane in aqueous solution, or a substituent which facilitates bonding of the dioxetane to a membrane, film, bead, polymer or polymerizable group, or a substituent which enhances the kinetics of the dioxetane enzyme degradation (b) contacting said 1,2-dioxetane compound with said sample containing said enzyme; whereupon said enzyme cleaves said enzymatically cleavable labile substituent from said 1,2-dioxetane compound to form a negatively charged substituent bonded to said 1,2-dioxetane compound, said negatively charged substituent causing said 1,2-dioxetane compound to decompose to form a luminescent substance comprising said fluorescent chromophone group; and (c) detecting said luminescent substance as an indication of the presence of said enzyme.

2. A method of claim 1 wherein X is **methoxy**, Y-Z is a meta phosphate-substituted phenoxy group, and said enzyme is acid or alkaline phosphatase.

3. A method of claim 1 wherein X is **methoxy**, Y-Z is a meta β-D-galactoside-substituted phenoxy group, and said enzyme is a galactosidase.

4. An assay method for detection of a member of a specific binding pair in a sample by means of an optically detectable reaction, comprising (1) reacting (a) an enzymatically cleavable **chemiluminescent** 1,2-dioxetane compound capable of producing light energy when decomposed, stable at room temperature before a bond by which an enzymatically cleavable labile substituent thereof is intentionally cleaved, represented by the formula: ##STR32## wherein T is carboxylic acid substituted or **methoxy** substituted adamantyl bonded to the dioxetane ring through a spiro linkage; Y is phenoxy or naphthyloxy; X is **methoxy**, ethoxy or propoxy, and Z is an enzyme-cleavable group selected from the group consisting of phosphate ester group, acetate ester group, carboxyl group, 1-phospho-2,3-diacylglyceride group, 1-thio-D-glucoside group, adenosine triphosphate **analog** group, adenosine diphosphate **analog** group, adenosine monophosphate **analog** group, adenosine **analog** group, α-D-galactoside group, β-D-galactoside group, α-D-glucoside, β-D-glucoside group, α-D-mannoside group, β-D-mannoside group, β-D-fructofuranoside group, β-D-glycosiduranate group, p-toluenesulfonyl-L-arginine ester group and a p-toluenesulfonyl-L-arginine amide group with (b) a sample comprising an enzyme bound to one of said specific binding pair if present in said sample, to form a reaction mixture, so that said enzyme cleaves said enzyme-cleavable group to yield an electron-rich moiety bonded to the dioxetane ring to cause the dioxetane to decompose to form a luminescent substance and (2) monitoring said reaction mixture to determine if light is released, wherein the release of light is indicative of the presence of said member of a specific binding pair in said sample.

5. An assay method of claim 4 wherein said specific binding pair comprises an antigen and an antibody.

6. An assay method of claim 4 wherein said specific binding pair comprises an enzyme and a 1,2-dioxetane compound containing a group cleavable by said enzyme.

7. An assay method of claim 6 wherein said enzyme-cleavable group comprises a galactopyranoside, and said enzyme comprises a galactosidase.

8. An assay method of claim 4, conducted using a solid matrix, wherein nonspecific binding to said matrix is blocked by pretreating said matrix with a polymeric quaternary ammonium salt.

water-soluble enhancing substance that increases specific light energy production above that produced in its absence.

10. An assay method of claim 9, wherein said water-soluble enchancing substance is serum albumin.

11. An assay method of claim 9, wherein said enhancing substance is a polymeric quaternary ammonium salt.

12. An assay method of claim 11, wherein said polymeric quaternary ammonium salt is poly(vinylbenzyltrimethylammonium chloride), poly[vinylbenzyl(benzyldimethylammonium chloride)] or poly[vinylbenzyl(tributylammonium chloride)].

13. An assay method of claim 9, wherein said enhancing substance comprises a positively charged polymeric quaternary ammonium salt and fluorescein capable of forming a ternary complex with the negatively charged substituent of said 1,2-dioxetane compound produced following enzyme-catalyzed decomposition of said 1,2-dioxetane compound, whereby energy transfer occurs between said negatively charged substituent and fluorescein and light energy is emitted by fluorescein.

14. An assay method of claim 12, wherein said polymeric quaternary ammonium salt is poly(vinylbenzyltrimethylammonium chloride), poly[vinylbenzyl(benzyldimethylammonium chloride)] or poly[vinylbenzyl(tributylammonium chloride)].

15. A hybridization assay method for detection of the presence of a nucleic acid selected from the group consisting of DNA, RNA and fragments thereof in a sample, comprising: 1) contacting nucleic acid in the sample with a labeled complementary **oligonucleotide** probe to form a hybridized pair, 2) contacting the hybridized pair with a molecule which binds strongly to the label of the **oligonucleotide**, said strongly binding molecule being conjugated with an enzyme capable of cleaving an enzymatically-cleavable 1,2-dioxetane to release light energy, 3) adding to said conjugated, hybridized pair an enzymatically cleavable 1,2-dioxetane substrate represented by the formula ##STR33## wherein T is carboxylic acid substituted or **methoxy** substituted adamantyl bonded to the dioxetane ring through a spiro linkage; Y is phenoxy or naphthyloxy; X is **methoxy**, ethoxy or propoxy, and Z is an enzyme-cleavable group selected from the group consisting of phosphate ester group, acetate ester group, carboxyl group, 1-phospho-2,3-diacylglyceride group, 1-thio-D-glucoside group, adenosine triphosphate **analog** group, adenosine diphosphate **analog** group, adenosine monophosphate **analog** group, adenosine **analog** group, α-D-galactoside group, β-D-galactoside group, α-D-glucoside, β-D-glucoside group, α-D-mannoside group, β-D-mannoside group, β-D-fructofuranoside group, β-D-glycosiduranate group, p-toluenesulfonyl-L-arginine ester group and a p-toluenesulfonyl-L-arginine amide group; to form a reaction mixture 4) monitoring said reaction mixture to detect the production of light wherein light production is indicative of the presence of said nucleic acid in said sample.

16. The hybridization assay method of claim 15, wherein said **oligonucleotide** probe binds to all of said nucleic acid.

17. The hybridization assay method of claim 15, wherein said **oligonucleotide** probe binds to a portion of said nucleic acid.

18. The assay method of claim 15, wherein the label of said **oligonucleotide** probe is biotin.

19. An assay method of claim 15 wherein the molecule capable of strong interaction with the label of the **oligonucleotide** is avidin or

streptavidin.

20. An assay method of claim 15 wherein the enzyme is an acid or alkaline phosphatase, X is **methoxy**, and Y-Z is a meta phosphate-substituted phenoxy group.

21. An assay method of claim 13 wherein the enzyme is a galactosidase, X is **methoxy**, and Y-Z is a meta β-D-galactoside-substituted phenoxy group.

22. An assay method of claim 15 wherein light energy is detected by light-sensitive film.

23. An assay method of claim 15 wherein light energy is detected by a photoelectric cell.

24. An assay method of claim 15 wherein said **oligonucleotide** probe is covalently labeled with an enzyme capable of decomposing said 1,2-dioxetane to emit light energy.

25. An assay method of claim 15 wherein said label on said **oligonucleotide** probe comprises a covalently bound antigen that is immunochemically bound to an antibody-enzyme conjugate, wherein said antibody is directed to said antigen and said enzyme is capable of decomposing said 1,2-dioxetane compound to emit light energy.

26. An assay method of either claim 24 or 25 wherein said enzyme is an acid or alkaline phosphatase, X is **methoxy**, and Y-Z is a meta phosphate-substituted phenoxy group.

27. An assay method of either claim 24 or 25 wherein said enzyme is a galactosidase, X is **methoxy**, and Y-Z is a meta β-D-galactoside-substituted phenoxy group.

28. An assay method of any one of claims 24 or 25 wherein the binding of said probe to said nucleic acid is carried out on a nylon membrane.

29. A kit for detecting a first substance in a sample, comprising (1) an enzymatically cleavable **chemiluminescent** 1,2-dioxetane compound capable of producing light energy when decomposed, stable at room temperature before a bond by which an enzymatically cleavable labile substituent thereof is intentionally cleaved, represented by the formula: ##STR34## wherein T is carboxylic acid substituted or **methoxy** substituted adamantyl bonded to the dioxetane ring through a spiro linkage; Y is phenoxy or naphthyloxy; X is **methoxy**, ethoxy or propoxy, and Z is an enzyme-cleavable group selected from the group consisting of phosphate ester group, acetate ester group, carboxyl group, 1-phospho-2,3-diacylglyceride group, 1-thio-D-glucoside group, adenosine triphosphate **analog** group, adenosine diphosphate **analog** group, adenosine monophosphate **analog** group, adenosine **analog** group, α-D-galactoside group, β-D-galactoside group, α-D-glucoside, β-D-glucoside group, α-D-mannoside group, β-D-mannoside group, β-D-fructofuranoside group, β-D-glycosiduranate group, p-toluenesulfonyl-L-arginine ester group and a p-toluenesulfonyl-L-arginine amide group; and (2) an enzyme capable of cleaving said enzyme cleavable group.

30. The kit of claim 29, wherein X is **methoxy**, Y-Z is a meta phosphate-substituted phenoxy group, and said enzyme is acid or alkaline phosphatase.

31. The kit of claim 29, wherein X is **methoxy**, Y-Z is a β-D-galactoside-substituted phenoxy group, and said enzyme is a galactosidase.

image-reproducing means for detecting said light energy.

33. The kit of claim 32, wherein said image-reproducing means is photographic film.

34. A kit for detecting a nucleic acid or fragment thereof in a sample by hybridization of said nucleic acid or fragment to a complementary labeled **oligonucleotide** probe, comprising (1) an enzymatically cleavable **chemiluminescent** 1,2-dioxetane compound capable of producing light energy when decomposed, stable at room temperature before a bond by which an enzymatically cleavable labile substituent thereof is intentionally cleaved, represented by the formula: ##STR35## wherein T is carboxylic acid substituted or **methoxy** substituted adamantyl bonded to the dioxetane ring through a spiro linkage; Y is phenoxy or naphthyloxy; X is **methoxy**, ethoxy or propoxy, and Z is an enzyme-cleavable group selected from the group consisting of phosphate ester group, acetate ester group, carboxyl group, 1-phospho-2,3-diacylglyceride group, 1-thio-D-glucoside group, adenosine triphosphate **analog** group, adenosine diphosphate **analog** group, adenosine monophosphate **analog** group, adenosine **analog** group, α-D-galactoside group, β-D-galactoside group, α-D-glucoside, β-D-glucoside group, α-D-mannoside group, β-D-mannoside group, β-D-fructofuranoside group, β-D-glycosiduranate group, p-toluenesulfonyl-L-arginine ester group and a p-toluenesulfonyl-L-arginine amide group; (2) a covalently enzyme-labeled **oligonucleotide** probe wherein said enzyme cleaves said enzyme-cleavable group; and, (3) a nylon membrane.

35. The kit of claim 34 wherein X is **methoxy**, Y-Z is a meta phosphate-substituted phenoxy group, and said enzyme is acid or alkaline phosphatase.

36. The kit of claim 34 wherein X is **methoxy**, Y-Z is a meta β-D-galactoside-substituted phenoxy group, and said enzyme is a galactosidase.

37. The kit of claim 34, further comprising image-reproducing means for detecting said light energy.

38. The kit of claim 37 wherein said image-reproducing means is photographic film.

39. A kit for detecting a nucleic acid or fragment thereof in a sample by hybridization of said nucleic acid or fragment to a complementary labeled **oligonucleotide** probe, comprising (1) an enzymatically cleavable **chemiluminescent** 1,2-dioxetane compound capable of producing light energy when decomposed, substantially stable at room temperature before a bond by which an enzymatically cleavable labile substituent thereof is intentionally cleaved, represented by the formula: ##STR36## wherein T is carboxylic acid substituted or **methoxy** substituted adamantyl bonded to the dioxetane ring through a spiro linkage; Y is phenoxy or naphthyloxy; X is **methoxy**, ethoxy or propoxy, and Z is an enzyme-cleavable group selected from the group consisting of phosphate ester group, acetate ester group, carboxyl group, 1-phospho-2,3-diacylglyceride group, 1-thio-D-glucoside group, adenosine triphosphate **analog** group, adenosine diphosphate **analog** group, adenosine monophosphate **analog** group, adenosine **analog** group, α-D-galactoside group, β-D-galactoside group, α-D-glucoside, β-D-glucoside group, α-D-mannoside group, β-D-mannoside group, β-D-fructofuranoside group, β-D-glycosiduranate group, p-toluenesulfonyl-L-arginine ester group and a p-toluenesulfonyl-L-arginine amide group; (2) a complementary **oligonucleotide** probe covalently labeled with biotin or a biotin derivative; avidin or streptavidin covalently bound to an enzyme which

cleaves said enzyme cleavable group; and, (4) a nylon membrane.

40. The kit of claim 39 wherein X is **methoxy**, Y-Z is a meta phosphate-substituted phenoxy group, and said enzyme is acid or alkaline phosphatase.

41. The kit of claim 39, wherein X is **methoxy**, Y-Z is a meta β-D-galactoside-substituted phenoxy group, and said enzyme is a galactosidase.

42. The kit of 39, further comprising image-reproducing means for detecting said light energy.

43. The kit of claim 42 wherein said image-reproducing means is photographic film.

44. A kit for detecting a nucleic acid or fragment thereof in a sample by hybridization of said nucleic acid or fragment to a complementary labeled **oligonucleotide** probe, comprising (1) an enzymatically cleavable **chemiluminescent** 1,2-dioxetane compound capable of producing light energy when decomposed, stable at room temperature before a bond by which an enzymatically cleavable labile substituent thereof is intentionally cleaved, represented by the formula: ##STR37## wherein T is carboxylic acid substituted or **methoxy** substituted adamantyl bonded to the dioxetane ring through a spiro linkage; Y is phenoxy or naphthyloxy; X is **methoxy**, ethoxy or propoxy, and Z is an enzyme-cleavable group selected from the group consisting of phosphate ester group, acetate ester group, carboxyl group, 1-phospho-2,3-diacylglyceride group, 1-thio-D-glucoside group, adenosine triphosphate **analog** group, adenosine diphosphate **analog** group, adenosine monophosphate **analog** group, adenosine **analog** group, α-D-galactoside group, β-D-galactoside group, α-D-glucoside, β-D-glucoside group, α-D-mannoside group, β-D-mannoside group, β-D-fructofuranoside group, β-D-glycosiduranate group, p-toluenesulfonyl-L-arginine ester group and a p-toluenesulfonyl-L-arginine amide group; (2) a complementary **oligonucleotide** probe covalently labeled with an antigen; (3) an antibody directed to said antigen covalently bound to an enzyme which cleaves said enzyme-cleavable group; and, (4) a nylon membrane.

45. The kit of claim 44 wherein said enzyme is an acid or alkaline phosphatase, X is **methoxy**, and Y-Z is a meta phosphate-substituted phenoxy group.

46. The kit of claim 44 wherein said enzyme is a galactosidase, X is **methoxy**, and Y-Z is a meta β-D-galactoside-substituted phenoxy group.

47. The kit of claim 44, further comprising image-reproducing means for detecting said light energy.

48. The kit of claim 47 wherein said image-reproducing means is photographic film.

49. The kit of any one of claims 29, 34, 39, 44, further comprising a water-soluble enhancing substance that increases specific light energy production above that produced in its absence.

50. The kit of claim 49 wherein said water-soluble enhancing substance is serum albumin.

51. The kit of claim 49 wherein said enhancing substance is a polymeric quaternary ammonium salt.

52. The kit of claim 51 wherein said polymeric quaternary ammonium salt

is poly(vinylbenzyltrimethylammonium chloride),
poly[vinylbenzyl(benzyldimethylammonium chloride)] or poly[vinylbenzyl(tributylammonium chloride)].

53. The kit of claim 49 wherein said enhancing substance comprises a positively charged polymeric quaternary ammonium salt and fluorescein capable of forming a ternary complex with the negatively charged product of said 1,2-dioxetane compound produced following enzyme-catalyzed decomposition of said 1,2-dioxetane compound, whereby energy transfer occurs between said negatively charged product and fluorescein and light energy is emitted by fluorescein.

54. The kit of claim 53 wherein said polymeric quaternary ammonium salt is poly(vinylbenzyltrimethylammonium chloride), poly[vinylbenzyl(benzyldimethylammonium chloride)] or poly[vinylbenzyl(tributylammonium chloride)].

55. A kit for detecting a protein in a sample, comprising an enzymatically cleavable **chemiluminescent** 1,2-dioxetane compound capable of producing light energy when decomposed, substantially stable at room temperature before a bond by which an enzymatically cleavable labile substituent thereof is intentionally cleaved, represented by the formula: ##STR38## wherein T is a cycloalkyl group or a fused polycyclo-alkylidene group bonded to the dioxetane ring through a spiro linkage; Y is a fluorescent chromophore capable of absorbing energy to form an excited energy state from which it emits optically detectable energy to return to its original energy state; X is hydrogen or an alkyl, aryl, aralkyl, alkaryl, heteroalkyl, heteroaryl, cycloalkyl or cycloheteroalkyl group, or an enzyme-cleavable group containing a bond cleavable by an enzyme to yield an electron-rich moiety bonded to the dioxetane ring, Z is hydrogen, hydroxyl or an enzyme-cleavable group containing a bond cleavable by an enzyme to yield an electron-rich moiety bonded to the dioxetane ring, at least one of X and Z being an enzyme-cleavable group, and T also includes a substituent which enhances the solubility of the dioxetane in aqueous solution, or a substituent which facilitates bonding of the dioxetane to a membrane, film, bead, polymer or polymerizable group, or a substituent which enhances the kinetics of the dioxetane enzyme degradation; an antibody directed to said protein covalently bound to an enzyme capable of decomposing said 1,2-dioxetane compound to emit light energy; and, a membrane upon which protein-antibody binding is conducted.

56. The kit of claim 55 wherein said membrane is a nylon or nitrocellulose membrane.

57. The kit of claim 55 wherein X is **methoxy**, Y-Z is a meta phosphonate-substituted phenoxy group, and said enzyme is acid or alkaline phosphatase.

58. The kit of claim 55 wherein X is **methoxy**, Y-Z is a meta β-D-galactoside-substituted phenoxy group, and said enzyme is a galactosidase.

59. The kit of any one of claims 55-58 inclusive, further comprising image-reproducing means for detecting said light energy.

60. The kit of claim 59 wherein said image reproducing means is photographic film.

61. A kit for detecting a protein in a sample comprising an enzymatically cleavable **chemiluminescent** 1,2-dioxetane compound capable of producing light energy when decomposed, substantially stable at room temperature before a bond by which an enzymatically cleavable labile substituent thereof is intentionally cleaved, represented by the formula: ##STR39## wherein T is a cycloalkyl group or a fused polycyclo-alkylidene group bonded to the dioxetane ring through a spiro

form an excited energy state from which it emits optically detectable energy to return to its original energy state; X is hydrogen or an alkyl, aryl, aralkyl, alkaryl, heteroalkyl, heteroaryl, cycloalkyl or cycloheteroalkyl group, or an enzyme-cleavable group containing a bond cleavable by an enzyme to yield an electron-rich moiety bonded to the dioxetane ring, Z is hydrogen, hydroxyl or an enzyme-cleavable group containing a bond cleavable by an enzyme to yield an electron-rich moiety bonded to the dioxetane ring, at least one of X and Z being an enzyme-cleavable group, and T also includes a substituent which enhances the solubility of the dioxetane in aqueous solution, or a substituent which facilitates bonding of the dioxetane to a membrane, film, bead, polymer or polymerizable group, or a substituent which enhances the kinetics of the dioxetane enzyme degradation; a first antibody directed to said protein; and, a second antibody directed to said first antibody covalently bound to an enzyme capable of decomposing said 1,2-dioxetane compound.

62. The kit of claim 61 wherein X is **methoxy,** Y-Z is a meta phosphate-substituted phenoxy group, and said enzyme is acid or alkaline phosphatase.

63. The kit of claim 61 wherein X is **methoxy,** Y-Z is a meta β-D-galactoside-substituted phenoxy group, and said enzyme is a galactosidase.

DRWD FIGS. 1-3 compare the total luminescence emissions obtained from each of AMPPD and its A-methoxyadamant-2'-ylidene and B-methoxyadamant-2'-ylidene **analogs,** respectively, obtained as described in Example IV below.

DRWD FIGS. 4-6 show TSH, RLU v. TSH for each of AMPPD and its A-methoxyadamant-2'-ylidene and B-methoxyadamant-2'-ylidene **analogs,** respectively, obtained as described in Example VIII below.

DRWD FIGS. 7A and 7B show the sensitivities of AMPPD and its A-methoxyadamant-2'-ylidene **analog** in the detection of an alkaline phosphate label in Herpes Simplex Virus I DNA by the method described in Example. . . .

DETD . . . to alkaline phosphatase. For another example, in DNA sequencing alkaline phosphatase--avidin binds to a biotinylated nucleotide base. Thereafter, an AMPPD **analog** of this invention is added to the gel or membrane filter. After short incubation, light is emitted as the result. . .

DETD The sensitivities of AMPPD and its 5'-A-methoxy **analog** in the detection of an alkaline phosphate label in Herpes Simplex Virus I DNA by the Snap® probe hybridization assay. . .

1. A method of detecting an enzyme in a sample comprising the steps of: (a) providing an enzymatically cleavable **chemiluminescent** 1,2-dioxetane compound capable of producing light energy when decomposed, substantially stable at room temperature before a bond by which an. . .

2. A method of claim 1 wherein X is **methoxy,** Y-Z is a meta phosphate-substituted phenoxy group, and said enzyme is acid or alkaline phosphatase.

3. A method of claim 1 wherein X is **methoxy,** Y-Z is a meta β-D-galactoside-substituted phenoxy group, and said enzyme is a galactosidase.

. . . . specific binding pair in a sample by means of an optically detectable reaction, comprising (1) reacting (a) an enzymatically cleavable **chemiluminescent** 1,2-dioxetane compound capable of producing light energy when decomposed, stable at room temperature before a bond by which an enzymatically cleavable labile substituent thereof is intentionally cleaved, represented by the formula: ##STR32## wherein T is carboxylic acid substituted or **methoxy** substituted adamantyl bonded to the dioxetane ring through a spiro linkage; Y is phenoxy or

naphthyloxy; X is **methoxy**, ethoxy or propoxy, and Z is an enzyme-cleavable group selected from the group consisting of phosphate ester group, acetate ester group, carboxyl group, 1-phospho-2,3-diacylglyceride group, 1-thio-D-glucoside group, adenosine triphosphate **analog** group, adenosine diphosphate **analog** group, adenosine monophosphate **analog** group, adenosine **analog** group, α-D-galactoside group, β-D-galactoside group, α-D-glucoside, β-D-glucoside group, α-D-mannoside group, β-D-mannoside group, β-D-fructofuranoside group, β-D-glycosiduranate group, p-toluenesulfonyl-L-arginine ester group and. . .

. . . . DNA, RNA and fragments thereof in a sample, comprising: 1) contacting nucleic acid in the sample with a labeled complementary **oligonucleotide** probe to form a hybridized pair, 2) contacting the hybridized pair with a molecule which binds strongly to the label of the **oligonucleotide**, said strongly binding molecule being conjugated with an enzyme capable of cleaving an enzymatically-cleavable 1,2-dioxetane to release light energy, 3). . . conjugated, hybridized pair an enzymatically cleavable 1,2-dioxetane substrate represented by the formula ##STR33## wherein T is carboxylic acid substituted or **methoxy** substituted adamantyl bonded to the dioxetane ring through a spiro linkage; Y is phenoxy or naphthyloxy; X is **methoxy**, ethoxy or propoxy, and Z is an enzyme-cleavable group selected from the group consisting of phosphate ester group, acetate ester group, carboxyl group, 1-phospho-2,3-diacylglyceride group, 1-thio-D-glucoside group, adenosine triphosphate **analog** group, adenosine diphosphate **analog** group, adenosine monophosphate **analog** group, adenosine **analog** group, α-D-galactoside group, β-D-galactoside group, α-D-glucoside, β-D-glucoside group, α-D-mannoside group, β-D-mannoside group, β-D-fructofuranoside group, β-D-glycosiduranate group, p-toluenesulfonyl-L-arginine ester group and. . .

16. The hybridization assay method of claim 15, wherein said **oligonucleotide** probe binds to all of said nucleic acid.

17. The hybridization assay method of claim 15, wherein said **oligonucleotide** probe binds to a portion of said nucleic acid.

18. The assay method of claim 15, wherein the label of said **oligonucleotide** probe is biotin.

19. An assay method of claim 15 wherein the molecule capable of strong interaction with the label of the **oligonucleotide** is avidin or streptavidin.

20. An assay method of claim 15 wherein the enzyme is an acid or alkaline phosphatase, X is **methoxy**, and Y-Z is a meta phosphate-substituted phenoxy group.

21. An assay method of claim 13 wherein the enzyme is a galactosidase, X is **methoxy**, and Y-Z is a meta β-D-galactoside-substituted phenoxy group.

24. An assay method of claim 15 wherein said **oligonucleotide** probe is covalently labeled with an enzyme capable of decomposing said 1,2-dioxetane to emit light energy.

25. An assay method of claim 15 wherein said label on said **oligonucleotide** probe comprises a covalently bound antigen that is immunochemically bound to an antibody-enzyme conjugate, wherein said antibody is directed to. . .

. . . . An assay method of either claim 24 or 25 wherein said enzyme is an acid or alkaline phosphatase, X is **methoxy**, and Y-Z is a meta phosphate-substituted phenoxy group.

galactosidase, X is **methoxy**, and Y-Z is a meta β-D-galactoside-substituted phenoxy group.

29. A kit for detecting a first substance in a sample, comprising (1) an enzymatically cleavable **chemiluminescent** 1,2-dioxetane compound capable of producing light energy when decomposed, stable at room temperature before a bond by which an enzymatically cleavable labile substituent thereof is intentionally cleaved, represented by the formula: ##STR34## wherein T is carboxylic acid substituted or **methoxy** substituted adamantyl bonded to the dioxetane ring through a spiro linkage; Y is phenoxy or naphthyloxy; X is **methoxy**, ethoxy or propoxy, and Z is an enzyme-cleavable group selected from the group consisting of phosphate ester group, acetate ester group, carboxyl group, 1-phospho-2,3-diacylglyceride group, 1-thio-D-glucoside group, adenosine triphosphate **analog** group, adenosine diphosphate **analog** group, adenosine monophosphate **analog** group, adenosine **analog** group, α-D-galactoside group, β-D-galactoside group, α-D-glucoside, β-D-glucoside group, α-D-mannoside group, β-D-mannoside group, β-D-fructofuranoside group, β-D-glycosiduranate group, p-toluenesulfonyl-L-arginine ester group and. . .

30. The kit of claim 29, wherein X is **methoxy**, Y-Z is a meta phosphate-substituted phenoxy group, and said enzyme is acid or alkaline phosphatase.

31. The kit of claim 29, wherein X is **methoxy**, Y-Z is a β-D-galactoside-substituted phenoxy group, and said enzyme is a galactosidase.

. . . nucleic acid or fragment thereof in a sample by hybridization of said nucleic acid or fragment to a complementary labeled **oligonucleotide** probe, comprising (1) an enzymatically cleavable **chemiluminescent** 1,2-dioxetane compound capable of producing light energy when decomposed, stable at room temperature before a bond by which an enzymatically cleavable labile substituent thereof is intentionally cleaved, represented by the formula: ##STR35## wherein T is carboxylic acid substituted or **methoxy** substituted adamantyl bonded to the dioxetane ring through a spiro linkage; Y is phenoxy or naphthyloxy; X is **methoxy**, ethoxy or propoxy, and Z is an enzyme-cleavable group selected from the group consisting of phosphate ester group, acetate ester group, carboxyl group, 1-phospho-2,3-diacylglyceride group, 1-thio-D-glucoside group, adenosine triphosphate **analog** group, adenosine diphosphate **analog** group, adenosine monophosphate **analog** group, adenosine **analog** group, α-D-galactoside group, β-D-galactoside group, α-D-glucoside, β-D-glucoside group, α-D-mannoside group, β-D-mannoside group, β-D-fructofuranoside group, β-D-glycosiduranate group, p-toluenesulfonyl-L-arginine ester group and a p-toluenesulfonyl-L-arginine amide group; (2) a covalently enzyme-labeled **oligonucleotide** probe wherein said enzyme cleaves said enzyme-cleavable group; and, (3) a nylon membrane.

35. The kit of claim 34 wherein X is **methoxy**, Y-Z is a meta phosphate-substituted phenoxy group, and said enzyme is acid or alkaline phosphatase.

36. The kit of claim 34 wherein X is **methoxy**, Y-Z is a meta β-D-galactoside-substituted phenoxy group, and said enzyme is a galactosidase.

. . . nucleic acid or fragment thereof in a sample by hybridization of said nucleic acid or fragment to a complementary labeled **oligonucleotide** probe, comprising (1) an enzymatically cleavable **chemiluminescent** 1,2-dioxetane compound capable of producing light energy when

decomposed, substantially stable at room temperature before a bond by which an. . . enzymatically cleavable labile substituent thereof is intentionally cleaved, represented by the formula: ##STR36## wherein T is carboxylic acid substituted or **methoxy** substituted adamantyl bonded to the dioxetane ring through a spiro linkage; Y is phenoxy or naphthyloxy; X is **methoxy**, ethoxy or propoxy, and Z is an enzyme-cleavable group selected from the group consisting of phosphate ester group, acetate ester group, carboxyl group, 1-phospho-2,3-diacylglyceride group, 1-thio-D-glucoside group, adenosine triphosphate **analog** group, adenosine diphosphate **analog** group, adenosine monophosphate **analog** group, adenosine **analog** group, α-D-galactoside group, β-D-galactoside group, α-D-glucoside, β-D-glucoside group, α-D-mannoside group, β-D-mannoside group, β-D-fructofuranoside group, β-D-glycosiduranate group, p-toluenesulfonyl-L-arginine ester group and a p-toluenesulfonyl-L-arginine amide group; (2) a complementary **oligonucleotide** probe covalently labeled with biotin or a biotin derivative; avidin or streptavidin covalently bound to an enzyme which cleaves said. . .

40. The kit of claim 39 wherein X is **methoxy**, Y-Z is a meta phosphate-substituted phenoxy group, and said enzyme is acid or alkaline phosphatase.

41. The kit of claim 39, wherein X is **methoxy**, Y-Z is a meta β-D-galactoside-substituted phenoxy group, and said enzyme is a galactosidase.

. . . . nucleic acid or fragment thereof in a sample by hybridization of said nucleic acid or fragment to a complementary labeled **oligonucleotide** probe, comprising (1) an enzymatically cleavable **chemiluminescent** 1,2-dioxetane compound capable of producing light energy when decomposed, stable at room temperature before a bond by which an enzymatically cleavable labile substituent thereof is intentionally cleaved, represented by the formula: ##STR37## wherein T is carboxylic acid substituted or **methoxy** substituted adamantyl bonded to the dioxetane ring through a spiro linkage; Y is phenoxy or naphthyloxy; X is **methoxy**, ethoxy or propoxy, and Z is an enzyme-cleavable group selected from the group consisting of phosphate ester group, acetate ester group, carboxyl group, 1-phospho-2,3-diacylglyceride group, 1-thio-D-glucoside group, adenosine triphosphate **analog** group, adenosine diphosphate **analog** group, adenosine monophosphate **analog** group, adenosine **analog** group, α-D-galactoside group, β-D-galactoside group, α-D-glucoside, β-D-glucoside group, α-D-mannoside group, β-D-mannoside group, β-D-fructofuranoside group, β-D-glycosiduranate group, p-toluenesulfonyl-L-arginine ester group and a p-toluenesulfonyl-L-arginine amide group; (2) a complementary **oligonucleotide** probe covalently labeled with an antigen; (3) an antibody directed to said antigen covalently bound to an enzyme which cleaves. . .

45. The kit of claim 44 wherein said enzyme is an acid or alkaline phosphatase, X is **methoxy**, and Y-Z is a meta phosphate-substituted phenoxy group.

46. The kit of claim 44 wherein said enzyme is a galactosidase, X is **methoxy**, and Y-Z is a meta β-D-galactoside-substituted phenoxy group.

55. A kit for detecting a protein in a sample, comprising an enzymatically cleavable **chemiluminescent** 1,2-dioxetane compound capable of producing light energy when decomposed, substantially stable at room temperature before a bond by which an. . .

57. The kit of claim 55 wherein X is **methoxy**, Y-Z is a meta phosphonate-substituted phenoxy group, and said enzyme is acid or alkaline phosphatase.

60. The kit of claim 59 wherein X is **methoxy**, Y-Z is a meta β-D-galactoside-substituted phenoxy group, and said enzyme is a galactosidase.

61. A kit for detecting a protein in a sample comprising an enzymatically cleavable **chemiluminescent** 1,2-dioxetane compound capable of producing light energy when decomposed, substantially stable at room temperature before a bond by which an. . .
62. The kit of claim 61 wherein X is **methoxy**, Y-Z is a meta phosphate-substituted phenoxy group, and said enzyme is acid or alkaline phosphatase.

63. The kit of claim 61 wherein X is **methoxy**, Y-Z is a meta β-D-galactoside-substituted phenoxy group, and said enzyme is a galactosidase.

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=> s us5554516/pn
L14          1 US5554516/PN

=> d l14,cbib,ab,clm
```

L14 ANSWER 1 OF 1 USPATFULL on STN
96:82591 Nucleic acid sequence amplification method, composition and kit.
Kacian, Daniel L., San Diego, CA, United States
McAllister, Diane L., San Diego, CA, United States
McDonough, Sherrol H., San Diego, CA, United States
Dattagupta, Nanibhushan, San Diego, CA, United States
Gen-Probe Incorporated, San Diego, CA, United States (U.S. corporation)
US 5554516 19960910 <--
APPLICATION: US 1993-162836 19931202 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method, composition and kit for amplifying a target nucleic acid sequence under conditions of substantially constant temperature, ionic strength, and pH and using only a single promoter-primer. To effect the amplification, a supply of a single promoter-primer having a promoter and a primer complementary to the 3'-end of the target sequence, and a reverse transcriptase and an RNA polymerase are provided to a mixture including the target sequence; the amplification proceeds accordingly. The invention is useful for generating copies of a nucleic acid target sequence for purposes that include assays to quantitate specific nucleic acid sequences in clinical, environmental, forensic and similar samples, cloning and generating probes.

CLM What is claimed is:
1. A method of amplifying a target ribonucleic acid sequence comprising the following steps: a) incubating a mixture comprising: a target nucleic acid comprising said target ribonucleic acid sequence, one or more promoter-primers comprising a single nucleic acid sequence comprising a promoter recognizable by an RNA polymerase and a primer located 3' relative to said promoter, said primer being sufficiently complementary to said target nucleic acid to form a promoter-primer:target nucleic acid complex at or near the 3'-end of said target ribonucleic acid sequence, and able to be extended to form a complement of said target ribonucleic acid sequence by a DNA polymerase, said DNA polymerase, and said RNA polymerase, at a temperature and in a solution effective to allow amplification of said target ribonucleic acid sequence, said mixture lacking a primer which forms a hybrid with said complement of said target ribonucleic acid sequence; and b) producing multiple copies of an RNA sequence complementary to said target ribonucleic acid sequence using said target ribonucleic acid sequence as a template.

2. The method of claim 1 wherein said DNA polymerase is a reverse transcriptase.

3. The method of claim 1 or 2 wherein said incubation is at essentially constant temperature.

4. The method of claim 1 or 2 wherein said target ribonucleic acid sequence and said one or more promoter-primers are incubated together prior to addition of said DNA polymerase and said RNA polymerase.

5. The method of claim 1 or 2 wherein said solution further comprises RNAse H activity.

6. The method of claim 1 or 2, wherein said solution further comprises an agent to create a definition at a 5'-end of said target ribonucleic acid sequence such that an extension reaction involving said target ribonucleic acid sequence will stop at said definition.

7. The method of claim 6, wherein said agent comprises a defining nucleic acid sequence sufficiently complementary to said 5'-end of said target ribonucleic acid sequence to be able to complex with said 5'-end of said target ribonucleic acid at said temperature and in said solution.

8. The method of claim 1 or 2, wherein said target nucleic acid comprises nucleotides at its 3'-end that are not within said promoter-primer:target nucleic acid complex.

9. The method of claim 1 or 2, wherein said 3'-end of said target nucleic acid is generated by chemical or enzymatic degradation or processing.

10. The method of claim 9 wherein said chemical or enzymatic degradation or processing comprises treatment with an exonuclease.

11. The method of claim 1 or 2, wherein said mixture further comprises one or more helper oligonucleotides.

12. The method of claim 1 or 2 wherein said RNA polymerase is a DNA-dependent RNA polymerase.

13. The method of claim 12 wherein said DNA-dependent RNA polymerase is selected from the group consisting of T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase.

14. The method of claim 1 or 2, wherein said mixture is screened by hybridization with a probe after said incubation.

15. A method of amplifying a target ribonucleic acid sequence comprising the steps of: a) incubating a mixture consisting essentially of: a target nucleic acid comprising said target ribonucleic acid sequence, a supply of promoter-primers comprising a single nucleic acid sequence comprising a promoter recognizable by an RNA polymerase and a primer located 3' relative to said promoter, said primer being sufficiently complementary to said target ribonucleic acid to form a promoter-primer:target nucleic acid complex at or near the 3'-end of said target ribonucleic acid sequence, said supply comprising one or more modified promoter-primers and one or more unmodified promoter-primers, wherein the ratio of said one or more modified promoter-primers to said one or more unmodified promoter-primers is effective to produce greater amplification compared to said one or more modified promoter-primers or said one or more unmodified promoter primers alone, a reverse transcriptase, and said RNA polymerase, at a temperature and in a solution effective to allow amplification of said target ribonucleic acid sequence, said incubating comprising essentially constant temperature during said amplification; and b) producing multiple copies of an RNA sequence complementary to said target ribonucleic sequence using said target ribonucleic acid sequence as a

template.

16. The method of claim 15, wherein said solution further comprises an agent which defines a 5'-end of said target ribonucleic acid sequence such that any extension reaction involving said target ribonucleic acid sequence will stop at said definition.

17. The method of claim 15 wherein said target nucleic acid comprises nucleotides located 3' of said promoter-primer:target nucleic acid complex.

18. The method of claim 15 wherein said reverse transcriptase is AMV or MMLV reverse transcriptase.

19. The method of claim 15 or 18, wherein each of said one or more modified promoter-primers independently have a modification selected from the group consisting of, 3' terminal phosphorothioate deoxyribonucleotide, non-nucleotide linkage, 3'-alkane-diol residue, and 3'-cordycepin.

20. The method of claim 15, wherein said mixture further comprises one or more helper oligonucleotides.

21. A method for amplifying a target ribonucleic acid sequence, comprising the steps of: a) contacting said target ribonucleic acid sequence with a plurality of promoter-primers comprising a single nucleic acid sequence comprising a promoter recognizable by an RNA polymerase and a primer located 3' relative to said promoter, said primer being able to complex at or near a 3'-end of said target ribonucleic acid sequence, and wherein one or more of said plurality of promoter-primers is an unmodified promoter-primer and one or more of said plurality of promoter-primers is a modified promoter-primer comprising a modified nucleotide at its 3'-end to prevent or decrease a nucleic acid extension reaction from proceeding therefrom, under conditions effective to allow said amplifying; and b) producing multiple copies of an RNA sequence complementary to said target ribonucleic acid sequence.

22. A composition for amplifying a target ribonucleic acid sequence using said target ribonucleic acid sequence as a template comprising: said target ribonucleic acid sequence, one or more promoter-primers comprising a single nucleic acid sequence comprising a promoter recognizable by an RNA polymerase and a primer located 3' relative to said promoter, said primer being sufficiently complementary to said target ribonucleic acid sequence to form a complex at or near the 3'-end of said target ribonucleic acid sequence, a reverse transcriptase, said RNA polymerase, and a solution of reagents able to allow amplification of said target ribonucleic acid sequence at essentially constant temperature; wherein a primer able to hybridize to a nucleic acid sequence complementary to said target sequence is not present.

23. The composition of claim 22 further comprising a defining oligonucleotide sufficiently complementary to a 5'-end of said target nucleic acid sequence to form a complex with said 5'-end of said target nucleic acid sequence at said temperature and in said solution.

24. The composition of 22 wherein said target ribonucleic acid sequence is present on RNA which comprises nucleotides located 3' of said complex.

25. The composition of claim 22 wherein said reverse transcriptase is AMV or MMLV reverse transcriptase.

26. The composition of claim 22 further comprising one or more helper oligonucleotides.

from the group consisting of T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase.

28. Nucleic acid consisting of a sequence chosen from the group consisting of: SEQ ID No 6, SEQ ID No 8, and SEQ ID No 9.

29. The method of claim 2 wherein said incubating is performed in the presence of one or more of DMSO and glycerol.

30. The method of claim 5, wherein said target nucleic acid comprises nucleotides located 3' of said promoter-primer:target nucleic acid complex.

31. A method of amplifying a target ribonucleic acid sequence comprising the following steps: a) incubating a mixture comprising: a target nucleic acid comprising said target ribonucleic acid sequence, one or more promoter-primers comprising a single nucleic acid sequence comprising a promoter recognizable by an RNA polymerase and a primer located 3' relative to said promoter, said primer being sufficiently complementary to said target nucleic acid to form a promoter-primer:target nucleic acid complex at or near the 3'-end of said target ribonucleic acid sequence, a DNA polymerase, and said RNA polymerase, at a temperature and in a solution effective to allow amplification of said target ribonucleic acid sequence, wherein said mixture lacks a primer which forms a hybrid with said complement of said target ribonucleic acid sequence; and b) producing multiple copies of an RNA sequence complementary to said target ribonucleic acid sequence using said target ribonucleic acid sequence as a template; wherein at least one of said one or more promoter-primers is a modified promoter-primer comprising a modification at its 3'-end to prevent or decrease a nucleic acid extension reaction from proceeding therefrom.

32. The method of claim 31, wherein said DNA polymerase is a reverse transcriptase.

33. The method of claim 32, wherein said one or more promoter-primers comprise one or more unmodified promoter-primers.

34. The method of claim 32, wherein said one or more promoter-primers comprises one or more modified promoter-primers and one or more unmodified promoter-primers, wherein said one or more modified promoter-primers and said one or more unmodified promoter-primers are present in a ratio of between about 150:1 and about 1:1, respectively.

35. The method of any of claims 31-34, wherein said modification is selected from the group consisting of, one or more ribonucleotide, 3' terminal phosphorothioate deoxyribonucleotide, nonnucleotide linkage, 3'-alkane-diol residue, and 3'-cordycepin.

36. The method of claim 32 or 33, wherein said reverse transcriptase is either AMY or MMLV reverse transcriptase.

37. The method claim 36, wherein said modification is selected from the group consisting of, one or more ribonucleotide, 3' terminal phosphorothioate deoxyribonucleotide, nonnucleotide linkage, 3'-alkane-diol residue, and 3'-cordycepin.

38. A composition for amplifying a target ribonucleic acid sequence using said target ribonucleic acid sequence as a template comprising: said target ribonucleic acid sequence, and one or more promoter-primers comprising a single nucleic acid sequence comprising a promoter recognizable by an RNA polymerase and a primer located 3' relative to said promoter, said primer being sufficiently complementary to said target ribonucleic acid sequence to form a complex at or near the 3'-end of said target ribonucleic acid sequence, a reverse transcriptase, said

target ribonucleic acid sequence at essentially constant temperature; wherein a primer able to hybridize to a nucleic acid sequence complementary to said target ribonucleic acid sequence is not present; wherein said one or more promoter-primers comprises one or more modified promoter-primers and one or more unmodified promoter-primers, said one or more modified promoter-primers and said one or more unmodified promoter primers being present in a ratio effective to produce amplification.

39. The composition of claim 38, wherein said ratio of one more modified promoter-primers to one or more unmodified promoter-primers is between about 150:1 and about 1:1, respectively.

40. The composition of claim 38 or 39, wherein each of said one or more modified promoter-primers have a modification selected from the group consisting of, one or more ribonucleotide, 3' terminal phosphorothioate deoxyribonucleotide, non-nucleotide linkage, 3'-alkane-diol residue, and 3'-cordycepin.

41. The composition of claim 40, wherein said modification is said 3'-alkane-diol residue.

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=> us5840488/pn
US5840488 IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s us5840488/pn
L15          1 US5840488/PN

=> d l15,cbib,ab,clm
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L15 ANSWER 1 OF 1 USPATFULL on STN
1998:147211 Nucleic acid probes for detection and/or quantitation of non-viral organisms.
Hogan, James John, San Diego, CA, United States
Gen-Probe Incorporated, San Diego, CA, United States (U.S. corporation)
US 5840488 19981124 <--
APPLICATION: US 1995-471394 19950606 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for preparing probes, as well as several probes for use in qualitative or quantitative hybridization assays are disclosed. The method comprises constructing an oligonucleotide that is sufficiently complementary to hybridize to a region of rRNA selected to be unique to a non-viral organism or group of non-viral organisms sought to be detected, said region of rRNA being selected by comparing one or more variable region rRNA sequences of said non-viral organism or group of non-viral organisms with one or more variable region rRNA sequences from one or more non-viral organisms sought to be distinguished. Hybridization assay probes for Mycobacterium avium, Mycobacterium intracellulare, the Mycobacterium tuberculosis-complex bacteria, Mycoplasma pneumoniae, Legionella, Salmonella, Chlamydia trachomatis, Campylobacter, Proteus mirabilis, Enterococcus, Enterobacter cloacae, E. coli, Pseudomonas group I, Neisseria gonorrhoeae, bacteria, and fungi also are disclosed.

CLM What is claimed is:
1. A method of making an oligonucleotide probe able to distinguish between a non-viral target species and a non-viral non-target species comprising the following steps: a) identifying one or more potential variable regions present in rRNA, or encoding rDNA, by comparing the rRNA or encoding rDNA of at least two different species belonging to the

same non-viral genus, wherein each of said one or more potential variable regions is identified based on at least a one base difference between the rRNA or encoding rDNA of said at least two different species in each of said one or more potential variable regions, wherein said at least two different species are not made up of both said target species and said non-target species; b) selecting a variable region from said potential variable regions identified in said step (a) by comparing the rRNA, or encoding rDNA, of said target species and said non-target species in one or more locations corresponding to said potential variable regions and identifying said variable region from said potential variable regions based on at least a one base difference in the rRNA, or encoding rDNA, of said target species and said non-target species; c) producing said oligonucleotide probe to comprise a target-complementary sequence, wherein said target-complementary sequence is obtained by substantially maximizing complementarity to said variable region present in said target species, while substantially minimizing complementarity to said variable region present in said non-target species, such that a duplex formed between said oligonucleotide probe and nucleic acid of said target species has a higher T_m than a duplex formed between said oligonucleotide probe and nucleic acid of said non-target species.

2. The method of claim 1, wherein said steps (a) and (b) are carried out using rRNA.

3. The method of claim 2, wherein said target and said non-target species belong to the same non-viral genus.

4. The method of claim 2, wherein said target and said non-target species belong to a different non-viral genus.

5. The method of claim 2, wherein said identifying one or more potential variable regions is carried out by comparing rRNA of at least three different species belonging to said non-viral genus.

6. The method of claim 2, wherein in each of said potential variable regions identified in said step (a) there is greater than a one base difference in 10 contiguous bases of rRNA of said at least two different species.

7. A method of making an oligonucleotide probe able to distinguish between a non-viral target species and a non-viral non-target species comprising the following steps: a) identifying one or more potential variable regions present in rRNA, or encoding rDNA, by comparing the rRNA or encoding rDNA of at least two different species having at least 90% rRNA homology to each other, wherein each of said one or more potential variable regions is identified based on at least a one base difference between the rRNA or encoding rDNA of said at least two different species in each of said one or more potential variable regions, wherein said at least two different species are not made up of both said target species and said non-target species; b) selecting for a variable region from said potential variable regions identified in said step (a) by comparing the rRNA, or encoding rDNA, of said target species and said non-target species in one or more locations corresponding to said potential variable regions and identifying said variable region from said potential variable regions based on at least a one base difference in the rRNA, or encoding rDNA, of said target species and said non-target species; c) producing said oligonucleotide probe to comprise a target-complementary sequence, wherein said target-complementary sequence is obtained by substantially maximizing complementarity to said variable region present in said target species, while substantially minimizing complementarity to said variable region present in said non-target species, such that a duplex formed between said oligonucleotide probe and nucleic acid of said target species has a higher T_m than a duplex formed between said oligonucleotide probe and nucleic acid of said non-target species.

8. The method of claim 7, wherein said steps (a) and (b) are carried out using rRNA.

9. The method of claim 8, wherein said target and said non-target species belong to a different non-viral genus.

10. The method of claim 8, wherein in each of said potential variable regions identified in said step (a) there is greater than a one base difference in 10 contiguous bases of rRNA of said first and said second species.

11. A method of making an oligonucleotide probe able to distinguish between a non-viral target species and a non-viral non-target species comprising the following steps: a) identifying one or more potential variable regions present in rRNA, or encoding rDNA, of at least two different species belonging to the same non-viral genus, wherein each of said one or more potential variable regions is identified based on at least a one base difference between the rRNA, or encoding rDNA, of said at least two different species in each of said one or more potential variable regions, and said at least two different species do not contain both said target species and said non-target species; b) selecting a variable region from said potential variable regions identified in said step (a) based on at least a one base difference in the rRNA, or encoding rDNA, of said target species and said non-target species; and c) producing said oligonucleotide probe to distinguish said variable region present in said target species from said variable region present in said non-target species, wherein said oligonucleotide probe comprises a non-radioactive label.

```
=> s us5639604/pn
L16          1 US5639604/PN

=> s us5658737/pn
L17          1 US5658737/PN

=> d l17,cbib,ab,clm

L17  ANSWER 1 OF 1  USPATFULL on STN
97:73448 Compositions and methods for the simultaneous detection and
    quantification of multiple specific nucleic acid sequences.
    Nelson, Norman Charles, San Diego, CA, United States
    Woodhead, James Stuart, Raglan, Great Britain
    Weeks, Ian, Cardiff, Great Britain
    Cheikh, Azzouz Ben, Del Mar, CA, United States
    Gen-Probe Incorporated, San Diego, CA, United States (U.S. corporation)
    US 5658737 19970819                                            <--
    APPLICATION: US 1996-692610  19960806 (8)
    DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
```

AB The invention relates to methods for simultaneously or sequentially detecting multiple nucleic acid analytes in a single medium utilizing oligonucleotide hybridization probes coupled to different chemiluminescent labeling reagents. The methods may be used in a heterogeneous, homogeneous or non-homogeneous assay system. The invention also relates to specific combinations of chemiluminescent labeling reagents suitable, when coupled to an oligonucleotide probe, for use together in methods for the detection of multiple nucleic acid analytes. The invention also concerns kits useful in these methods.

CLM What is claimed is:

1. A kit for the detection of two or more nucleic acid analytes suspected of being present in a sample, comprising, in one or more container: a) at least two different oligonucleotide hybridization assay probes, each able to specifically hybridize to a different nucleic acid

to each said probe, wherein each chemiluminescent label in said kit is chosen so that the chemiluminescence emitted by said label upon induction of light emission is separately distinguishable on the basis of wavelength of light emission, kinetics of the chemiluminescent reaction, or pH of reaction from the chemiluminescence emitted by at least one other of said labels; and said coupled labels are able to react with a destabilizing agent which will selectively destroy or inhibit the chemiluminescent potential of said labels when said probe has not formed a stable nucleic acid hybrid with an analyte without extinguishing the chemiluminescent potential of said labels coupled to hybridized probe; and the rate of destruction or inhibition of at least one hybrid-unassociated coupled label by said destabilizing agent is no more than 50 fold the rate of destruction or inhibition of at least one other of said hybrid-unassociated coupled labels by said agent.

2. The kit of claim 1 in which at least one of said chemiluminescent labels is an acridinium ester derivative.

3. The kit of claim 1 in which at least two of said chemiluminescent labels are acridinium ester derivatives.

4. The kit of claim 3 in which one of said acridinium esters is selected from the group: a) standard AE, b) naphthyl AE, c) o-diBr AE, d) 1- and 3- Me AE, e) 4,5-diMe AE, f) 2,7-diMe AE, g) o-Me AE, h) o-MeO(cinnamyl) AE, i) o-MeO AE, j) ortho AE, k) o-F-AE, l) 1- and 3-Me-o-F AE, m) 2,7-diMe-o-F AE, and n) 1- and 3-Me-m-diF AE.

5. The kit of claim 3 wherein the acridinium ester labels are chosen so that the wavelength of light emitted from at least one of said labels after initiation of a chemiluminescent reaction is sufficiently different from the wavelength of light emitted from at least one other said label to permit each label's separate detection in a single sample.

6. The kit of claim 5 wherein said acridinium ester derivatives are 2,7-diMe AE and standard AE.

7. The kit of claim 3 wherein the acridinium ester labels are chosen so that the chemiluminescent reaction kinetics of at least one said label is sufficiently different from the reaction kinetics of at least one other said label to permit each label's separate detection in a single sample.

8. The kit of claim 7 in which a first acridinium ester is standard AE and a second acridinium ester is selected from the group: naphthyl AE, o-diBr AE, o-diMe AE, o-Me AE, o-MeO (cinnamyl) AE, o-MeO AE, o-AE, o-F AE, 1-Me-o-F AE, and 1-Me-m-diF AE.

9. The kit of claim 7 in which a first acridinium ester is naphthyl AE and a second acridinium ester is selected from the group: 1-Me AE, 4,5-diMe AE, 2,7-di Me AE, o-Me AE, o-MeO (cinnamyl) AE, and o-AE.

10. The kit of claim 7 in which a first acridinium ester is o-diBr AE and a second acridinium ester is selected from the group: 1-Me AE, 4,5-diMe AE, 2,7-diMe AE, o-diMe AE, o-Me AE, o-MeO (cinnamyl) AE, and o-AE.

11. The kit of claim 7 in which a first acridinium ester is 1-Me AE and a second acridinium ester is selected from the group: o-diMe AE, o-Me AE, o-MeO (cinnamyl) AE, o-MeO AE, o-AE, o-F AE, 1-Me-o-F AE, and 1-Me-m-diF AE.

12. The kit of claim 7 in which a first acridinium ester is 4,5-diMe AE and a second acridinium ester is selected from the group: o-diMe AE, o-Me AE, o-MeO (cinnamyl) AE, o-MeO AE, o-AE, o-F AE, 1-Me-o-F AE, and 1-Me-m-diF AE.

13. The kit of claim 7 in which a first acridinium ester is 2,7-diMe AE and a second acridinium ester is selected from the group: o-diMe AE, o-Me AE, o-MeO (cinnamyl) AE, o-MeO AE, o-AE, o-F AE, 1-Me-o-F AE, and 1-Me-m-diF AE.

14. The kit of claim 7 in which a first acridinium ester is o-diMe AE and a second acridinium ester is selected from the group: o-Me AE, o-MeO (cinnamyl) AE, o-MeO AE, o-AE, o-F AE, 1-Me-o-F AE, and 1-Me-m-diF AE.

15. The kit of claim 7 in which a first acridinium ester is o-Me AE and a second acridinium ester is selected from the group: o-MeO (cinnamyl) AE, o-MeO AE, o-AE, o-F AE, 1-Me-o-F AE, and 1-Me-m-diF AE.

16. The kit of claim 7 in which a first acridinium ester is o-MeO (cinnamyl) AE and a second acridinium ester is selected from the group: o-MeO AE, o-F AE, 1Me-o-F AE, and 1-Me-m-diF AE.

17. The kit of claim 7 in which a first acridinium ester is o-MeO AE and a second acridinium ester is o-AE.

18. The kit of claim 7 in which a first acridinium ester is o-AE and a second acridinium ester is selected from the group consisting of o-F AE, 1-Me-o-F AE, and 1-Me-m-diF AE.

19. The kit of claim 3 in which said acridinium ester labels are chosen so that the optimal pH for the chemiluminescent reaction of at least one said coupled label is sufficiently different from that of at least one other said coupled label that the light emitted by each of said labels present in a single solution, following initiation of a chemiluminescent reaction, can be separately detected by adjustment of the solution from a first pH to one or more different pH.

20. The kit of claim 19 in which a first acridinium ester label is selected from the group consisting of: a) o-diBr AE, and b) o-Fl AE, and a second acridinium ester label is selected from the group consisting of c) standard AE, and d) o-MeO AE.

21. The kit of claim 1 in which all said chemiluminescent labels are acridinium ester derivatives.

22. The kit of claim 1 further comprising at least five said chemiluminescent labels, in which said at least five chemiluminescent labels are acridinium ester derivatives, and said two or more said acridinium ester derivatives are selected from the group consisting of: a) o-diBr AE b) 2,7-diMe AE c) o-MeO(cinnamyl) AE, d) o-Me AE, and e) o-diMe AE.

23. The kit of claim 1 further comprising at least five said chemiluminescent labels, in which said at least five chemiluminescent labels are acridinium ester derivatives, and said two or more said acridinium ester derivatives are selected from the group consisting of: a) o-diBr AE, b) 1-Me AE, c) o-AE, d) o-Me AE, and e) o-diMe AE.

24. The kit of claim 1 further comprising at least seven said chemiluminescent labels, in which said at least seven chemiluminescent labels are acridinium ester derivatives, and said two or more said acridinium ester derivatives are selected from the group consisting of: a) o-diBr AE b) 2,7-diMe AE c) 1-Me AE, d) o-AE, c) o-MeO(cinnamyl) AE, d) o-Me AE, and e) o-diMe AE.

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=> s us5770369/pn
L18          1 US5770369/PN
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L18 ANSWER 1 OF 1 USPATFULL on STN
1998:72403 Nucleic acid mediated electron transfer.

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Fraser, Scott E., Newport Beach, CA, United States
California Institute of Technology, Pasadena, CA, United States (U.S. corporation)

US 5770369 19980623 <--
APPLICATION: US 1996-660534 19960607 (8)
DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides for the selective covalent modification of nucleic acids with redox active moieties such as transition metal complexes. Electron donor and electron acceptor moieties are covalently bound to the ribose-phosphate backbone of a nucleic acid at predetermined positions. The resulting complexes represent a series of new derivatives that are bimolecular templates capable of transferring electrons over very large distances at extremely fast rates. These complexes possess unique structural features which enable the use of an entirely new class of bioconductors and photoactive probes.

CLM What is claimed is:

1. A composition comprising a single-stranded nucleic acid containing at least one electron donor moiety and at least one electron acceptor moiety, wherein said electron donor moiety and said electron acceptor moiety are covalently attached to said nucleic acid, and wherein said electron donor moiety and said electron acceptor moiety are not redox proteins.

2. A composition comprising a single-stranded nucleic acid containing at least one electron donor moiety and at least one electron acceptor moiety, wherein at least one of said electron donor moiety and said electron acceptor moiety is an electrode and the other is an electron transfer moiety which is not a redox protein and which is covalently attached to said nucleic acid.

3. A composition comprising a first single stranded nucleic acid containing at least one electron donor moiety and a second single stranded nucleic acid containing at least one electron acceptor moiety, wherein said electron donor and acceptor moieties are covalently attached to said nucleic acid such that there are no more than seven sigma bonds between each of said electron donor and acceptor moieties and the closest base, and wherein said electron donor moiety and said electron acceptor moiety are not redox proteins.

4. A composition comprising a first single stranded nucleic acid containing at least one electron donor moiety and a second single stranded nucleic acid containing at least one electron acceptor moiety, wherein at least one of said electron donor moiety and said electron acceptor moiety is an electrode, and the other is an electron transfer moiety which is not a redox protein and which is covalently attached to said nucleic acid.

5. A composition according to claim 1, 2, 3 or 4 wherein at least one of said electron acceptor moiety and said electron donor moiety is attached to a terminal base.

6. A composition according to claim 1, 2, 3 or 4 wherein said nucleic acid comprises a ribose-phosphate backbone.

7. A composition according to claim 6 wherein at least one of said electron acceptor moiety and said electron donor moiety is attached to a ribose of said ribose-phosphate backbone.

8. A composition according to claim 1, 2, 3 or 4 wherein said nucleic

acid comprises a nucleic acid analog.

9. A composition according to claim 8 wherein said nucleic acid analog comprises a peptide nucleic acid.

10. A composition according to claim 1, 2, 3 or 4 wherein at least one of said electron donor moiety and said electron acceptor moiety is a transition metal complex.

11. A composition according to claim 10 wherein said transition metal complex comprises ruthenium, rhenium, osmium, platinium, copper or iron.

12. A composition according to claim 1, 2, 3 or 4 wherein at least one of said electron donor moiety and said electron acceptor moiety is an organic electron donor or acceptor.

13. A composition according to claim 1 or 3 wherein at least one of said electron donor moiety and said electron acceptor moiety is an electrode.

14. A method of detecting a target sequence in a nucleic acid sample comprising: a) hybridizing a single-stranded nucleic acid according to claim 1 or 2 to said target sequence, if present, to form a hybridization complex; b) detecting electron transfer between said electron donor and said electron acceptor moieties.

15. A method of detecting a target sequence in a nucleic acid wherein said target sequence comprises a first target domain and a second target domain adjacent to said first target domain, wherein said method comprises: a) hybridizing a first nucleic acid containing at least one electron donor moiety to said first target domain; b) hybridizing a second nucleic acid containing at least one electron acceptor moiety to said second target domain, wherein said electron donor and electron acceptor moieties are covalently attached to said nucleic acid and are not redox proteins; and c) detecting electron transfer between said electron donor and said electron acceptor moieties.

16. A method of detecting a target sequence in a nucleic acid wherein said target sequence comprises a first target domain and a second target domain adjacent to said first target domain wherein said method comprises: a) hybridizing a first nucleic acid containing at least one electron donor moiety to said first target domain; b) hybridizing a second nucleic acid containing at least one electron acceptor moiety to said second target domain, wherein at least one of said electron donor moiety and said electron acceptor moiety is an electrode, and the other is an electron transfer moiety which is not a redox protein and which is covalently attached to said nucleic acid; and c) detecting electron transfer between said electron donor and said electron acceptor moieties.

17. A method according to claim 14, 15 or 16 wherein at least one of said electron acceptor moiety and said electron donor moiety is attached to a terminal base.

18. A method according to claim 14, 15 or 16 wherein said nucleic acid comprises a ribose-phosphate backbone.

19. A method according to claim 18 wherein at least one of said electron acceptor moiety and said electron donor moiety is attached to a ribose of said ribose-phosphate backbone.

20. A method according to claim 14, 15 or 16 wherein said nucleic acid comprises a nucleic acid analog.

21. A composition according to claim 20 wherein said nucleic acid analog comprises a peptide nucleic acid.

said electron donor moiety and said electron acceptor moiety is a transition metal complex.

23. A composition according to claim 22 wherein said transition metal complex comprises ruthenium, rhenium, osmium, platinium, copper or iron.

24. A method according to claim 14, 15 or 16 wherein at least one of said electron donor moiety and said electron acceptor moiety is an organic electron donor or acceptor.

25. A method according to claim 14, 15 or 16 wherein at least one of said electron donor moiety and said electron acceptor moiety is an electrode.

26. A method for making a single-stranded nucleic acid containing at least one covalently attached electron donor moiety and at least one covalently attached electron acceptor moiety, wherein said donor and acceptor moieties are not redox proteins, said method comprising: a) optionally attaching at least one nucleoside to a solid support to form a nascent nucleic acid; b) attaching a first modified nucleoside to said solid support or said nascent nucleic acid; c) attaching at least one additional nucleoside to said first modified nucleoside; d) attaching a second modified nucleoside to said nascent nucleic acid; e) optionally attaching at least one additional nucleoside to said nascent nucleic acid; and f) covalently attaching an electron donor moiety and an electron acceptor moiety to said modified nucleosides to form said single stranded nucleic acid.

27. A method for making a composition comprising a first single stranded nucleic acid containing at least one electron donor moiety and a second single stranded nucleic acid containing at least one electron acceptor moiety, wherein said electron donor and acceptor moieties are covalently attached to said nucleic acid such that there are no more than seven sigma bonds between each of said electron donor and acceptor moieties and the closest base, and wherein said electron donor moiety and said electron acceptor moiety are not redox proteins, said method comprising: a) making said first nucleic acid by: i) optionally attaching at least one nucleoside to a solid support to form a nascent first nucleic acid; ii) attaching a first modified nucleoside to said solid support or said nascent first nucleic acid; iii) attaching at least one additional nucleoside to said first modified nucleoside; iv) attaching a second modified nucleoside to said nascent first nucleic acid; v) optionally attaching at least one additional nucleoside to said nascent first nucleic acid; b) making said second nucleic acid by: i) optionally attaching at least one nucleoside to a solid support to form a nascent second nucleic acid; ii) attaching a first modified nucleoside to said solid support or said second nascent nucleic acid; iii) attaching at least one additional nucleoside to said first modified nucleoside iv) attaching a second modified nucleoside to said nascent second nucleic acid; v) optionally attaching at least one additional nucleoside to said nascent second nucleic acid; and c) covalently attaching an electron donor moiety and an electron acceptor moiety to said modified nucleosides.

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L19          1 US5554516/PN

=> d l19,cbib,ab,clm

L19  ANSWER 1 OF 1  USPATFULL on STN
96:82591 Nucleic acid sequence amplification method, composition and kit.
    Kacian, Daniel L., San Diego, CA, United States
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Dattagupta, Nanibhushan, San Diego, CA, United States
Gen-Probe Incorporated, San Diego, CA, United States (U.S. corporation)
US 5554516 19960910 <--
APPLICATION: US 1993-162836 19931202 (8)
DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method, composition and kit for amplifying a target nucleic acid sequence under conditions of substantially constant temperature, ionic strength, and pH and using only a single promoter-primer. To effect the amplification, a supply of a single promoter-primer having a promoter and a primer complementary to the 3'-end of the target sequence, and a reverse transcriptase and an RNA polymerase are provided to a mixture including the target sequence; the amplification proceeds accordingly. The invention is useful for generating copies of a nucleic acid target sequence for purposes that include assays to quantitate specific nucleic acid sequences in clinical, environmental, forensic and similar samples, cloning and generating probes.

CLM What is claimed is:

1. A method of amplifying a target ribonucleic acid sequence comprising the following steps: a) incubating a mixture comprising: a target nucleic acid comprising said target ribonucleic acid sequence, one or more promoter-primers comprising a single nucleic acid sequence comprising a promoter recognizable by an RNA polymerase and a primer located 3' relative to said promoter, said primer being sufficiently complementary to said target nucleic acid to form a promoter-primer:target nucleic acid complex at or near the 3'-end of said target ribonucleic acid sequence, and able to be extended to form a complement of said target ribonucleic acid sequence by a DNA polymerase, said DNA polymerase, and said RNA polymerase, at a temperature and in a solution effective to allow amplification of said target ribonucleic acid sequence, said mixture lacking a primer which forms a hybrid with said complement of said target ribonucleic acid sequence; and b) producing multiple copies of an RNA sequence complementary to said target ribonucleic acid sequence using said target ribonucleic acid sequence as a template.

2. The method of claim 1 wherein said DNA polymerase is a reverse transcriptase.

3. The method of claim 1 or 2 wherein said incubation is at essentially constant temperature.

4. The method of claim 1 or 2 wherein said target ribonucleic acid sequence and said one or more promoter-primers are incubated together prior to addition of said DNA polymerase and said RNA polymerase.

5. The method of claim 1 or 2 wherein said solution further comprises RNAse H activity.

6. The method of claim 1 or 2, wherein said solution further comprises an agent to create a definition at a 5'-end of said target ribonucleic acid sequence such that an extension reaction involving said target ribonucleic acid sequence will stop at said definition.

7. The method of claim 6, wherein said agent comprises a defining nucleic acid sequence sufficiently complementary to said 5'-end of said target ribonucleic acid sequence to be able to complex with said 5'-end of said target ribonucleic acid at said temperature and in said solution.

8. The method of claim 1 or 2, wherein said target nucleic acid comprises nucleotides at its 3'-end that are not within said promoter-primer:target nucleic acid complex.

9. The method of claim 1 or 2, wherein said 3'-end of said target

nucleic acid is generated by chemical or enzymatic degradation or processing.

10. The method of claim 9 wherein said chemical or enzymatic degradation or processing comprises treatment with an exonuclease.

11. The method of claim 1 or 2, wherein said mixture further comprises one or more helper oligonucleotides.

12. The method of claim 1 or 2 wherein said RNA polymerase is a DNA-dependent RNA polymerase.

13. The method of claim 12 wherein said DNA-dependent RNA polymerase is selected from the group consisting of T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase.

14. The method of claim 1 or 2, wherein said mixture is screened by hybridization with a probe after said incubation.

15. A method of amplifying a target ribonucleic acid sequence comprising the steps of: a) incubating a mixture consisting essentially of: a target nucleic acid comprising said target ribonucleic acid sequence, a supply of promoter-primers comprising a single nucleic acid sequence comprising a promoter recognizable by an RNA polymerase and a primer located 3' relative to said promoter, said primer being sufficiently complementary to said target ribonucleic acid to form a promoter-primer:target nucleic acid complex at or near the 3'-end of said target ribonucleic acid sequence, said supply comprising one or more modified promoter-primers and one or more unmodified promoter-primers, wherein the ratio of said one or more modified promoter-primers to said one or more unmodified promoter-primers is effective to produce greater amplification compared to said one or more modified promoter-primers or said one or more unmodified promoter primers alone, a reverse transcriptase, and said RNA polymerase, at a temperature and in a solution effective to allow amplification of said target ribonucleic acid sequence, said incubating comprising essentially constant temperature during said amplification; and b) producing multiple copies of an RNA sequence complementary to said target ribonucleic sequence using said target ribonucleic acid sequence as a template.

16. The method of claim 15, wherein said solution further comprises an agent which defines a 5'-end of said target ribonucleic acid sequence such that any extension reaction involving said target ribonucleic acid sequence will stop at said definition.

17. The method of claim 15 wherein said target nucleic acid comprises nucleotides located 3' of said promoter-primer:target nucleic acid complex.

18. The method of claim 15 wherein said reverse transcriptase is AMV or MMLV reverse transcriptase.

19. The method of claim 15 or 18, wherein each of said one or more modified promoter-primers independently have a modification selected from the group consisting of, 3' terminal phosphorothioate deoxyribonucleotide, non-nucleotide linkage, 3'-alkane-diol residue, and 3'-cordycepin.

20. The method of claim 15, wherein said mixture further comprises one or more helper oligonucleotides.

21. A method for amplifying a target ribonucleic acid sequence, comprising the steps of: a) contacting said target ribonucleic acid sequence with a plurality of promoter-primers comprising a single nucleic acid sequence comprising a promoter recognizable by an RNA

polymerase and a primer located 3' relative to said promoter, said primer being able to complex at or near a 3'-end of said target ribonucleic acid sequence, and wherein one or more of said plurality of promoter-primers is an unmodified promoter-primer and one or more of said plurality of promoter-primers is a modified promoter-primer comprising a modified nucleotide at its 3'-end to prevent or decrease a nucleic acid extension reaction from proceeding therefrom, under conditions effective to allow said amplifying; and b) producing multiple copies of an RNA sequence complementary to said target ribonucleic acid sequence.

22. A composition for amplifying a target ribonucleic acid sequence using said target ribonucleic acid sequence as a template comprising: said target ribonucleic acid sequence, one or more promoter-primers comprising a single nucleic acid sequence comprising a promoter recognizable by an RNA polymerase and a primer located 3' relative to said promoter, said primer being sufficiently complementary to said target ribonucleic acid sequence to form a complex at or near the 3'-end of said target ribonucleic acid sequence, a reverse transcriptase, said RNA polymerase, and a solution of reagents able to allow amplification of said target ribonucleic acid sequence at essentially constant temperature; wherein a primer able to hybridize to a nucleic acid sequence complementary to said target sequence is not present.

23. The composition of claim 22 further comprising a defining oligonucleotide sufficiently complementary to a 5'-end of said target nucleic acid sequence to form a complex with said 5'-end of said target nucleic acid sequence at said temperature and in said solution.

24. The composition of 22 wherein said target ribonucleic acid sequence is present on RNA which comprises nucleotides located 3' of said complex.

25. The composition of claim 22 wherein said reverse transcriptase is AMV or MMLV reverse transcriptase.

26. The composition of claim 22 further comprising one or more helper oligonucleotides.

27. The composition of claim 22 wherein said RNA polymerase is selected from the group consisting of T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase.

28. Nucleic acid consisting of a sequence chosen from the group consisting of: SEQ ID No 6, SEQ ID No 8, and SEQ ID No 9.

29. The method of claim 2 wherein said incubating is performed in the presence of one or more of DMSO and glycerol.

30. The method of claim 5, wherein said target nucleic acid comprises nucleotides located 3' of said promoter-primer:target nucleic acid complex.

31. A method of amplifying a target ribonucleic acid sequence comprising the following steps: a) incubating a mixture comprising: a target nucleic acid comprising said target ribonucleic acid sequence, one or more promoter-primers comprising a single nucleic acid sequence comprising a promoter recognizable by an RNA polymerase and a primer located 3' relative to said promoter, said primer being sufficiently complementary to said target nucleic acid to form a promoter-primer:target nucleic acid complex at or near the 3'-end of said target ribonucleic acid sequence, a DNA polymerase, and said RNA polymerase, at a temperature and in a solution effective to allow amplification of said target ribonucleic acid sequence, wherein said mixture lacks a primer which forms a hybrid with said complement of said target ribonucleic acid sequence; and b) producing multiple copies of an RNA sequence

ribonucleic acid sequence as a template; wherein at least one of said one or more promoter-primers is a modified promoter-primer comprising a modification at its 3'-end to prevent or decrease a nucleic acid extension reaction from proceeding therefrom.

32. The method of claim 31, wherein said DNA polymerase is a reverse transcriptase.

33. The method of claim 32, wherein said one or more promoter-primers comprise one or more unmodified promoter-primers.

34. The method of claim 32, wherein said one or more promoter-primers comprises one or more modified promoter-primers and one or more unmodified promoter-primers, wherein said one or more modified promoter-primers and said one or more unmodified promoter-primers are present in a ratio of between about 150:1 and about 1:1, respectively.

35. The method of any of claims 31-34, wherein said modification is selected from the group consisting of, one or more ribonucleotide, 3' terminal phosphorothioate deoxyribonucleotide, nonnucleotide linkage, 3'-alkane-diol residue, and 3'-cordycepin.

36. The method of claim 32 or 33, wherein said reverse transcriptase is either AMY or MMLV reverse transcriptase.

37. The method claim 36, wherein said modification is selected from the group consisting of, one or more ribonucleotide, 3' terminal phosphorothioate deoxyribonucleotide, nonnucleotide linkage, 3'-alkane-diol residue, and 3'-cordycepin.

38. A composition for amplifying a target ribonucleic acid sequence using said target ribonucleic acid sequence as a template comprising: said target ribonucleic acid sequence, and one or more promoter-primers comprising a single nucleic acid sequence comprising a promoter recognizable by an RNA polymerase and a primer located 3' relative to said promoter, said primer being sufficiently complementary to said target ribonucleic acid sequence to form a complex at or near the 3'-end of said target ribonucleic acid sequence, a reverse transcriptase, said RNA, and a solution of reagents able to allow amplification of said target ribonucleic acid sequence at essentially constant temperature; wherein a primer able to hybridize to a nucleic acid sequence complementary to said target ribonucleic acid sequence is not present; wherein said one or more promoter-primers comprises one or more modified promoter-primers and one or more unmodified promoter-primers, said one or more modified promoter-primers and said one or more unmodified promoter primers being present in a ratio effective to produce amplification.

39. The composition of claim 38, wherein said ratio of one more modified promoter-primers to one or more unmodified promoter-primers is between about 150:1 and about 1:1, respectively.

40. The composition of claim 38 or 39, wherein each of said one or more modified promoter-primers have a modification selected from the group consisting of, one or more ribonucleotide, 3' terminal phosphorothioate deoxyribonucleotide, non-nucleotide linkage, 3'-alkane-diol residue, and 3'-cordycepin.

41. The composition of claim 40, wherein said modification is said 3'-alkane-diol residue.

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=> s us5399491/pn
L20          1 US5399491/PN
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=> d l20,cbib,ab,clm

L20 ANSWER 1 OF 1 USPATFULL on STN
95:24837 Nucleic acid sequence amplification methods.
Kacian, Daniel L., San Diego, CA, United States
Fultz, Timothy J., Vista, CA, United States
Gen-Probe Incorporated, San Diego, CA, United States (U.S. corporation)
US 5399491 19950321 <--
APPLICATION: US 1992-855732 19920319 (7)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods of synthesizing multiple copies of a target nucleic acid sequence autocatalytically under conditions of substantially constant temperature, ionic strength, and pH are provided in which multiple RNA copies of the target sequence autocatalytically generate additional copies. These methods are useful for generating copies of a nucleic acid target sequence for purposes which include assays to quantitate specific nucleic acid sequences in clinical, environmental, forensic and similar samples, cloning and generating probes.

CLM What is claimed is:

1. A method of synthesizing multiple copies of a target nucleic acid sequence which consists essentially of: (a) treating a nucleic acid which comprises an RNA target sequence with a first oligonucleotide which comprises a first primer which has a hybridizing sequence sufficiently complementary to a 3'-terminal portion of the target sequence to hybridize therewith and which optionally has a sequence 5' to the hybridizing sequence which includes a promoter for an RNA polymerase, under conditions whereby an oligonucleotide/target sequence hybrid is formed and DNA synthesis may be initiated; (b) extending the primer in an extension reaction using the target as a template to give a DNA primer extension product complementary to the RNA target; (c) separating the DNA primer extension product from the RNA target using an enzyme which selectively degrades the RNA target; (d) treating the DNA primer extension product with a second oligonucleotide which comprises a primer or a splice template and which has a hybridizing sequence sufficiently complementary to the 3'-terminal portion of the target sequence to hybridize therewith, under conditions whereby an oligonucleotide/target sequence hybrid is formed and DNA synthesis may be initiated, provided that if the first oligonucleotide does not have a promoter, then the second oligonucleotide is a splice template which has a sequence 5' to the hybridizing sequence which includes a promoter for an RNA polymerase; (e) extending the 3'-terminus of either the second oligonucleotide or the first primer extension product, or both, in a DNA extension reaction to produce a template for an RNA polymerase; and (f) using the template of step (e) to produce multiple RNA copies of the target sequence using an RNA polymerase which recognizes the promoter sequence; wherein said method is conducted under conditions of constant temperature and wherein a reverse transcriptase comprising RNase H activity is used in the method, and no other enzyme comprising RNAse H activity is used in the method.

2. A method of synthesizing multiple copies of a target nucleic acid sequence which consists essentially of: (a) treating a target nucleic acid which comprises an RNA target sequence with a first oligonucleotide which comprises a first primer which has a hybridizing sequence sufficiently complementary to a 3'-terminal portion of the target sequence to hybridize therewith and a sequence 5' to the hybridizing sequence which includes a promoter for an RNA polymerase, under conditions whereby an oligonucleotide/target sequence hybrid is formed and DNA synthesis may be initiated; (b) extending the primer in an extension reaction using the target as a template to give a first DNA primer extension product complementary to the RNA target; (c) separating the first DNA primer extension product from the RNA target using an enzyme which selectively degrades the RNA target; (d) treating the first DNA primer extension product with a second oligonucleotide which

comprises a second primer which has a hybridizing sequence sufficiently complementary to the 3'-terminal portion of the target sequence to hybridize therewith, under conditions whereby an oligonucleotide/target sequence is formed and DNA synthesis may be initiated; (e) extending the 3'-terminus of the second primer in a DNA extension reaction to give a second DNA primer extension product, thereby producing a template for an RNA polymerase; and (f) using the template of step (e) to produce multiple RNA copies of the target sequence using an RNA polymerase which recognizes the promoter sequence; wherein said method is conducted under conditions of constant temperature and wherein a reverse transcriptase comprising RNase H activity is used in the method, and no other enzyme comprising RNase H activity is used in the method.

3. A method of synthesizing multiple copies of a target nucleic acid sequence which consists essentially of: (a) treating a nucleic acid which comprises an RNA target sequence with a first oligonucleotide which comprises a first primer which has a hybridizing sequence sufficiently complementary to a 3'-terminal portion of the target sequence to complex therewith, under conditions whereby an oligonucleotide/target sequence hybrid is formed and DNA synthesis may be initiated; (b) extending the 3' terminus of the first primer in an extension reaction using the target as a template to give a DNA primer extension product complementary to the RNA target; (c) separating the DNA primer extension product from the RNA target using an enzyme which selectively degrades the RNA target; (d) treating the DNA primer extension product with a second oligonucleotide which comprises a splice template which has a hybridizing sequence sufficiently complementary to the 3' terminus of the primer extension product to hybridize therewith and a sequence 5' to the hybridizing sequence which includes a promoter for an RNA polymerase, under conditions whereby an oligonucleotide/target sequence hybrid is formed and DNA synthesis may be initiated; (e) extending the 3'-terminus of the primer extension product to add thereto a sequence complementary to the promoter, thereby producing a template for an RNA polymerase; and (f) using the template of step (e) to produce multiple RNA copies of the target sequence using an RNA polymerase which recognizes the promoter; wherein said method is conducted under conditions of constant temperature and wherein a reverse transcriptase comprising RNase H activity is used in the method, and no other enzyme comprising. RNase H activity is used in the method.

4. A method of synthesizing multiple copies of a target sequence which consists essentially of: (a) treating a single stranded target nucleic acid which comprises a DNA target sequence having a defined 3' terminus with a first oligonucleotide which comprises a splice template which has a hybridizing sequence sufficiently complementary to a 3' terminal portion of the target sequence to hybridize therewith and a sequence 5' to the hybridizing sequence which includes a promoter for an RNA polymerase, under conditions whereby an oligonucleotide/target sequence hybrid is formed and DNA synthesis may be initiated; (b) extending the 3' terminus of the target to add a sequence complementary to the promoter, thereby producing a template for an RNA polymerase; and (c) using the template of step (b) to produce multiple RNA copies of the target sequence using an RNA polymerase which recognizes the promoter; wherein said method is conducted under conditions of constant temperature and wherein a reverse transcriptase comprising RNase H activity is used in the method, and no other enzyme comprising RNase H activity is used in the method.

5. A method of synthesizing multiple copies of a target nucleic acid sequence which consists essentially of: (a) treating a single stranded target nucleic acid which comprises a DNA target sequence with a first oligonucleotide which comprises a first primer which has a hybridizing sequence sufficiently complementary to a 3'-terminal portion of the target sequence to hybridize therewith and a sequence which includes a promoter for an RNA polymerase, under conditions whereby an oligonucleotide/target sequence hybrid is formed and DNA synthesis may

be initiated; (b) extending the primer in a DNA extension reaction using the target as a template to give a first DNA primer extension product complementary to the DNA target; (c) separating the first primer extension product from the target; (d) treating the first primer extension product with a second oligonucleotide which comprises a second primer which has a hybridizing sequence sufficiently complementary to the 3'-terminal portion of the target sequence to hybridize therewith, under conditions whereby an oligonucleotide/target sequence hybrid is formed and DNA synthesis may be initiated; (e) extending the 3' terminus of the second primer in a DNA extension reaction to give a second DNA primer extension product, thereby producing a template for a RNA polymerase; (f) using the template of step (e) to produce multiple RNA copies of the target sequence using an RNA polymerase which recognizes the promoter sequence; (g) treating an RNA copy from step (f) with the second primer under conditions whereby an oligonucleotide/target sequence hybrid is formed and DNA synthesis may be initiated; (h) extending the 3'-terminus of the second primer in a DNA extension reaction to give a second DNA primer extension product complementary to the RNA copy; (i) separating the DNA primer extension product from the RNA copy using an enzyme which selective degrades the RNA copy; (j) treating the second primer extension product with the first primer, under conditions whereby an oligonucleotide/target hybrid is formed and DNA synthesis may be initiated; (k) extending the 3'-terminus of the first primer in a DNA extension reaction to give a first DNA primer extension product and the 3'-terminus of the second primer extension product, thereby producing a template for an RNA polymerase; and (l) using the template of step (k) to produce multiple copies of the target sequence using an RNA polymerase which recognizes the promoter; wherein said steps (d) through (l) are conducted under conditions of constant temperature and wherein a reverse transcriptase comprising RNase H activity is used in the method, and no other enzyme comprising RNase H activity is used in the method.

6. A method of synthesizing multiple copies of a target nucleic acid sequence which consists essentially of: (a) treating a single stranded target nucleic acid which comprises a DNA target sequence with a first oligonucleotide which comprises a primer which has a hybridizing sequence sufficiently complementary to a 3'-terminal portion of the target sequence to hybridize therewith under conditions whereby an oligonucleotide/target sequence complex is formed and DNA synthesis may be initiated; (b) extending the 3'-terminus of the primer in an extension reaction using the target as a template to give a DNA primer extension product complementary to the target; (c) separating the primer extension product from the target; (d) treating the primer extension product with a second oligonucleotide which comprises a splice template which has a hybridizing sequence sufficiently complementary to the 3' terminal portion of the target sequence to hybridize therewith and a sequence 5' to the hybridizing sequence which includes a promoter for an RNA polymerase, under conditions whereby an oligonucleotide/target sequence hybrid is formed and DNA synthesis may be initiated; (e) extending the 3'-terminus of the primer extension product to add thereto a sequence complementary to the promoter, thereby producing a template for the RNA polymerase; and (f) using the template of step (e) to produce multiple RNA copies of the target sequence using an RNA polymerase which recognizes the promoter; wherein said steps (d) through (f) are conducted under conditions of constant temperature and wherein a reverse transcriptase comprising RNase H activity is used in the method, and no other enzyme comprising RNase H activity is used in the method.

7. A method for synthesizing multiple copies of a target nucleic acid sequence under conditions of constant temperature which consists essentially of: (a) combining (1) a target nucleic acid which comprises a single-stranded RNA target sequence; (2) a first oligonucleotide which comprises a primer which has a hybridizing sequence sufficiently complementary to a 3' terminal portion of the RNA target sequence to hybridize therewith and which optionally has a sequence 5' to the

hybridizing sequence which includes a promoter for an RNA polymerase; (3) a second oligonucleotide which comprises a primer or a splice template and which has a hybridizing sequence sufficiently complementary to the 3'-terminal portion of a complement to the RNA target sequence to hybridize therewith, provided that if the first oligonucleotide does not have a promoter, then the second oligonucleotide is a splice template which has a sequence 5' to the hybridizing sequence which includes a promoter for an RNA polymerase; (4) a DNA polymerase; (5) an enzyme which selectively degrades the RNA strand of an RNA:DNA duplex; and (6) an RNA polymerase which recognizes the promoter; and (b) incubating the mixture of step (a) under DNA priming and nucleic acid synthesizing conditions, which include constant temperature; and wherein a reverse transcriptase comprising RNase H activity is used in the method, and no other enzyme comprising RNase H activity is used in the method.

8. A method for synthesizing multiple copies of a target nucleic acid sequence under conditions of constant temperature which consists essentially of: (a) combining: (1) a target nucleic acid which comprises an RNA target sequence; (2) a primer and a splice template of opposite sense wherein one has a hybridizing sequence sufficiently complementary to a 3'-terminal portion of the target sequence of the RNA target to hybridize therewith and the other has a hybridizing sequence sufficiently complementary to the 3' terminal portion of the target sequence of its complement to hybridize therewith and wherein the splice template has a sequence 5' to the hybridizing sequence which includes a promoter; (3) a DNA polymerase: (4) an enzyme which selectively degrades the RNA strand of an RNA:DNA duplex; and (5) an RNA polymerase which recognizes the promoter of the splice template; and (b) incubating the mixture of step (a) under DNA priming and nucleic acid synthesizing conditions which include constant temperature; and wherein a reverse transcriptase comprising RNase H activity is used in the method, and no other enzyme comprising RNase H activity is used in the method.

9. A method for synthesizing multiple copies of a target nucleic acid sequence which consists essentially of: (a) combining: (1) a nucleic acid which comprises a single stranded DNA target sequence; (2) a first primer which has a hybridizing sequence sufficiently complementary to a 3'-terminal portion of the target sequence to hybridize therewith and a sequence 5' to the hybridizing sequence which includes a promoter for an RNA polymerase; (3) a DNA polymerase: (b) incubating the mixture of step (a) under DNA priming and synthesizing conditions whereby a primer extension product complementary to the target sequence is synthesized using the target sequence as a template; (c) treating the reaction mixture of step (b) to cause separation of DNA duplexes; (d) adding to the reaction mixture of step (c): (1) a second primer which has a hybridizing sequence sufficiently complementary to the 3'-terminal portion of the target sequence of the primer extension product to hybridize therewith; (2) a DNA polymerase (3) an enzyme which selectively degrades the RNA strand of an RNA:DNA complex; and (4) an RNA polymerase which recognizes the promoter; and (e) incubating the mixture of step (d) under DNA priming and nucleic acid synthesizing conditions which include constant temperature; and wherein a reverse transcriptase comprising RNase H activity is used in the method, and no other enzyme comprising RNase H activity is used in the method.

10. A method for synthesizing multiple copies of a target nucleic acid sequence under conditions of constant temperature which consists essentially of: (a) combining: (1) a nucleic acid which comprises a target sequence; (2) a first oligonucleotide which comprises first primer which has a hybridizing sequence sufficiently complementary to a 3' terminal portion of the target sequence to hybridize thereto and which optionally has a sequence 5' to the hybridizing sequence which includes a promoter for an RNA polymerase; (3) a second oligonucleotide which comprises a second primer or a splice template and which has a hybridizing sequence sufficiently complementary to the 3' terminal portion of the target sequence to hybridize thereto, provided that if

oligonucleotide is a splice template, which has a sequence 5' to the hybridizing sequence which includes a promoter; (4) a DNA polymerase (5) an enzyme which selectively degrades the RNA strand of an RNA: DNA duplex; and (6) an RNA polymerase; and (b) incubating the mixture of step (a) under DNA priming and nucleic acid synthesizing conditions, which include constant temperature; and wherein a reverse transcriptase comprising RNase H activity is used in the method, and no other enzyme comprising RNase H activity is used in the method.

11. A method according to claim 1, further comprising using the oligonucleotides and RNA copies to synthesize multiple copies of the target sequence.

12. A method according to claim 2, wherein the second primer has a sequence 5' to the hybridizing sequence which includes a promoter for an RNA polymerase.

13. A method according to claim 2, further comprising using the primers and RNA copies to synthesize multiple copies of the target sequence.

14. A method according to claim 13 wherein the second primer has a sequence 5' to the hybridizing sequence which includes a promoter for an RNA polymerase.

15. A method according to claim 2, further consisting essentially of: (g) treating an RNA copy from step (f) with the second primer under conditions whereby an oligonucleotide/target sequence hybrid is formed and DNA synthesis may be initiated. (h) extending the 3'-terminus of the second primer in a DNA extension reaction to give a second DNA primer extension product which is complementary to the RNA copy; (i) separating the second DNA primer extension product from the RNA copy using an enzyme which selectively degrades the RNA copy; (j) treating the second DNA primer extension product with the first primer under conditions whereby an oligonucleotide/target sequence hybrid is formed and DNA synthesis may be initiated. (k) extending the 3'-terminus of the first primer in a DNA extension reaction to give a first DNA primer extension product and the 3'-terminus of the second DNA primer extension product, thereby producing a template for a RNA polymerase; and (l) using the template of step (k) to produce multiple copies of the target sequence using an RNA polymerase which recognizes the promoter sequence; wherein said method is conducted under conditions of constant temperature and wherein a reverse transcriptase comprising RNase H activity is used in the method, and no other enzyme comprising RNase H activity is used in the method.

16. A method according to claim 15 wherein the second primer has a sequence 5' to the hybridizing sequence which includes a promoter for an RNA polymerase.

17. A method according to claim 15 further comprising using the primers and RNA copies to synthesize multiple copies of the target sequence.

18. A method according to claim 17 wherein the second primer has a sequence 5' to the hybridizing sequence which includes a promoter for an RNA polymerase.

19. A method according to claim 15 further consisting essentially of: (m) using the RNA copies of step (l), repeating steps (g) to (l) to synthesize multiple copies of the target sequence.

20. A method according to claim 19 wherein the second primer has a sequence 5' to the hybridizing sequence which includes a promoter for an RNA polymerase.

21. A method according to claim 2 further consisting essentially of: (g)

treating the RNA copies of step (f) with the first and second primers under hybridizing conditions; (h) extending the primers in a DNA extension reaction using the RNA copies as templates to give DNA primer extension products; (i) separating the DNA primer extension products from the RNA copies using an enzyme which selectively degrades the RNA copies; (j) treating the DNA primer extension products with the primers under hybridizing conditions; (k) extending the primers in a DNA extension reaction to give a complementary primer extension product, thereby producing templates for a RNA polymerase; and (l) using the templates of step (k) to produce multiple copies of the target sequence using an RNA polymerase which recognizes the promoter and wherein a reverse transcriptase comprising RNase H activity is used in the method, and no other enzyme comprising RNase H activity is used in the method.

22. A method according to claim 21 further consisting essentially of using the primers and RNA copies to synthesize multiple copies of the target sequence.

23. A method according to claim 21 further consisting essentially of: (m) using the RNA copies of step (l), repeating steps (g) to (l) to synthesize multiple copies of the target sequence.

24. A method according to claim 3, further consisting essentially of using the oligonucleotides and the RNA copies to synthesize multiple copies of the target sequence.

25. A method according to claim 3, further consisting essentially of: (g) using the RNA copies of step (f), repeating steps (a) to (f) to synthesize multiple copies of the target sequence.

26. A method according to claim 25 wherein the 3' terminus of the splice template is blocked.

27. A method according to claim 25 wherein in step (e), the splice template acts as a second primer and the 3' terminus of the splice template is extended in an DNA extension reaction to give a second primer extension product which is complementary to the first primer extension product.

28. A method according to claim 27 wherein the first primer has a sequence 5' to the hybridizing sequence which includes a promoter for an RNA polymerase.

29. A method according to claim 26 wherein said primer and said splice template comprise a single oligonucleotide having a sequence which comprises the splice template 5' to a sequence which comprises the primer.

30. A method according to claim 4, wherein the 3' terminus of the splice template is blocked.

31. A method according to claim 4, further consisting essentially of: (d) treating an RNA copy of step (c) with a second oligonucleotide which comprises a primer which has a hybridizing sequence sufficiently complementary to the 3' terminal portion of the RNA copy to complex therewith, under conditions whereby an oligonucleotide/target sequence hybrid is formed and DNA synthesis may be initiated; (e) extending the 3' terminus of the primer in a DNA extension reaction to give a DNA primer extension product which is complementary to the RNA copy; (f) separating the DNA primer extension product from the RNA copy; (g) treating the DNA primer extension product with the splice template under conditions whereby an oligonucleotide/target sequence hybrid is formed and DNA synthesis may be initiated; (h) extending the 3' terminus of the primer extension product in a DNA extension reaction to add a sequence complementary to the promoter, thereby producing a template for an RNA polymerase; and (i) using the template of step (h) to produce multiple

RNA copies of the target sequence using an RNA polymerase which recognizes the promoter and wherein a reverse transcriptase comprising RNase H activity is used in the method, and no other enzyme comprising RNase H activity is used in the method.

32. A method according to claim 31 further consisting essentially of using the oligonucleotides and the RNA copies of step (i), to synthesize multiple copies of the target sequence.

33. A method according to claim 32 wherein the 3' terminus of the splice template is blocked.

34. A method according to claim 31 further consisting essentially of: (j) using the RNA copies of step (i), repeating steps (d) to (i) to synthesize multiple copies of the target sequence.

35. A method according to claim 5, wherein the second primer has a sequence 5' to the hybridizing sequence which includes a promoter for an RNA polymerase.

36. A method according to claim 35 further consisting essentially of using the oligonucleotides and RNA copies to synthesize multiple copies of the target sequence.

37. A method according to claim 35 further consisting essentially of: (m) using the RNA copies of step (l) repeating steps (g) to (l) to synthesize multiple copies of the target sequence.

38. A method according to claim 6, further consisting essentially of: (g) treating an RNA copy of step (f) with the primer under conditions whereby an oligonucleotide/target sequence hybrid is formed and DNA synthesis may be initiated; (h) extending the 3'-terminus of the primer in a DNA extension reaction to give a primer extension product; (i) separating the primer extension from the RNA copy using an enzyme which selectively degrades the RNA copy; (j) treating the primer extension product with the splice template under conditions whereby an oligonucleotide/target sequence hybrid is formed and DNA synthesis may be initiated; (k) extending the 3' terminus of the primer extension product to add thereto a sequence complementary to the promoter, thereby producing a template for an RNA polymerase; (l) using the template of step (k) to produce RNA copies of the target sequence using an RNA polymerase which recognizes the promoter and wherein a reverse transcriptase comprising RNase H activity is used in the method, and no other enzyme comprising RNase H activity is used in the method.

39. A method according to claim 38 wherein the 3' terminus of the splice template is blocked.

40. A method according to claim 38 further consisting essentially of using the oligonucleotides and RNA copies to synthesize multiple copies of the target sequence.

41. A method according to claim 38 further consisting essentially of: (m) using the RNA copies of step (l), repeating steps (g) to (l) to synthesize multiple copies of the target sequence.

42. A method according to claims 7 or 41, wherein the first primer has a promoter.

43. A method according to claim 42 wherein the second oligonucleotide comprises a second primer which has a sequence 5' to the hybridizing sequence which includes a promoter for an RNA polymerase.

44. A method according to claim 43 wherein the DNA polymerase comprises said reverse transcriptase.

45. A method according to claim 10, wherein the first oligonucleotide has a promoter sequence.

46. A method according to claim 10 wherein the DNA polymerase comprises said reverse transcriptase.

47. A method according to claim 10 wherein the DNA polymerase comprises a reverse transcriptase.

48. The method of claim 29, wherein step (c) is performed without prior amplification of said template for an RNA polymerase.

49. The method of claim 6, wherein step (f) is performed without prior amplification of said template for an RNA polymerase.

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L21 ANSWER 1 OF 1 USPATFULL on STN
97:70657 Detecting or quantifying multiple analytes using labelling techniques.
Woodhead, James Stuart, Cardiff, United Kingdom
Weeks, Ian, Cardiff, United Kingdom
Batmanghelich, Shariar, Cardiff, United Kingdom
Gen Probe Incorporated, United States (U.S. corporation)
US 5656207 19970812 <--
APPLICATION: US 1995-439311 19950511 (8)
PRIORITY: GB 1989-14563 19890624
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for the assay, detection etc. of each of a plurality of substances of interest in a sample comprises labelling each of the substances with one or more components each capable of taking part in a respective distinguishable chemiluminescent reaction. Luminescent reagents for use in the method are also disclosed.

CLM What is claimed is:

1. A specific binding assay for two or more individual analytes in a liquid test sample which assay comprises: a) contacting the sample with two or more specific labeled reagents, wherein each such reagent comprises i) a member of a specific binding pair to which an individual analyte is a member, and ii) a different, specific light-emitting label covalently or non-covalently joined to said member, in order to form at least one labeled said specific binding pair, b) physically or chemically differentiating each said labeled specific binding pair from said labeled reagents, c) generating and measuring light emission from each specifically labeled specific binding pair, as a measure of the presence or amount of each said analyte, wherein each of the labels emit light at distinguishable emission maxima, or emit peak energy at distinguishable times after the generation of said emission, from at least one other of said labels.

2. The method of claim 1 wherein at least one of said labels is selected from the group consisting of acridinium compounds, phenanthridium compounds, phthalhydrazides, oxalate esters, dioxetanes and dioxetanones.

3. The method of claim 2 wherein at least one label forms an acridone before light is emitted from said label.

4. The method of claim 2 wherein at least one of said labels is an aryl acridinium ester.

5. The method of claim 4 wherein the aryl ring of at least one said

group, each independently selected from the group consisting of an electron-withdrawing group and an electron-donating group such that emission of light occurs over said distinguishable times.

6. The method of claim 4 wherein the acridinium ring of at least one said label or labels is substituted at one or more position with a chemical group, each independently selected from the group consisting of an electron-withdrawing group and an electron-donating group such that emission of light occurs over said distinguishable times.

7. The method of claim 5 or 6 wherein said electron-withdrawing group is a halogen.

8. The method of claim 5 or 6 wherein said electron-donating group is selected from the group consisting of a methyl group and a methoxy group.

9. The method of claim 2 wherein at least one of said labels is an optionally substituted diphenylanthracene.

10. The method of claim 1 wherein at least one of said labels is a fluorescent molecule which is able to emit light by energy transfer from another molecule.

11. The method of claim 1, 4, 5, or 6 wherein each said specific binding partner is selected from the group consisting of hormones, vitamins, co-factors, nucleic acids, antigens, antibodies, haptens, ligands, enzymes, and enzyme substrates.

12. The method of claim 1, 4, 5 or 6 wherein the labels comprised in at least two of said different specific labeled specific binding pairs are caused to emit light at substantially the same time.

13. The method of claim 1 or 4 wherein the intensity of the light emitted from said labeled specific binding pairs is detected over different time intervals which may overlap in relation to the starting point of said detection and the duration of said light emission.

14. The method of claim 1 or 4 wherein the light emitted from at least two said labeled specific binding pairs is detected over different wavelength ranges.

15. The method of claim 1 or 4 wherein said sample is contacted with at least three specific labeled reagents, wherein light emitted by labels joined to at least two said reagents can be distinguished by detection over different wavelength ranges, and light emitted by labels joined to at least two said reagents can be distinguished by variation of light intensity over time.

16. The method of claim 1 wherein said light emissions are filtered by different light filtering means, said means filtering light over different wavelength ranges, and the filtered light is detected.

17. The method of claim 4 wherein a first of said different labels is an acridinium ester substituted with electron withdrawing groups and a second of said different labels is an acridinium ester substituted with electron donating groups, and wherein, upon simultaneous induction of light emission from said labels, light emission from the first label occurs over a period of time in excess of light emission from said second label, wherein said groups are selected such that emission of light occurs over said distinguishable times.

18. The method of claim 4 wherein, upon induction of light emission from said labels, a first of said different labels is an acridinium ester which emits light at a first wavelength and a second of said different

conjugation than said first label, which emits light at a second wavelength longer than the first wavelength.

19. The method of claim 18 wherein said first label emits maximum light in a wavelength range of from 400 to 500 nm and said second label emits maximum light in a wavelength range of from 500 to 700 nm.

20. The method of claim 1 or 4 wherein each said label is covalently or non-covalently joined to said specific binding partner.

21. The method of claim 4 wherein two or more of said different labels are each an aryl acridinium ester.

22. The method of claim 4 wherein each said specifically labeled specific binding pairs comprise analyte bound to a specific binding partner by an interaction selected from the group consisting of an immunoassay binding reaction, a receptor binding reaction and a nucleic acid hybridization reaction.

23. The method of claim 1 wherein at least two of said specific labeled reagents individually comprise a chemiluminescent label.

24. A kit for the detection of more than one analyte in a test sample comprising two or more specifically labeled reagents, each said reagent comprising (i) a member of a specific binding pair to which an individual analyte is a member, and (ii) a different, specific label covalently or non-covalently joined to said binding pair member, said label being detectable by the emission of light from said label, wherein each of the labels emit light at separate and distinct emission maxima, or emit peak energy at separate and distinct times after the generation of said emission, from at least one other of said labels, and wherein upon contacting said reagents with a test sample containing said analytes, the presence or amount of each said analyte is determined after the initiation of light emission.

25. The kit of claim 24 wherein each of said labels is independently selected from the group consisting of acridinium compounds, phenanthridium-compounds, phthalhydrazides, oxalate esters, dioxetanes and dioxtaetanones.

26. The kit of claim 25 wherein two or more of said different labels are each an aryl acridinium ester.

27. The kit of claim 24 wherein said binding pair member is selected from the group consisting of a nucleic acid and a protein.

28. The kit of claim 26 wherein a first of said different labels is an acridinium ester substituted with electron withdrawing groups and a second of said different labels is an acridinium ester substituted with electron donating groups, and wherein, upon simultaneous induction of light emission from said labels, light emission from the first label occurs over a period of time in excess of light emission from said second label, wherein said groups are selected such that emission of light occurs over said distinguishable times.

29. The kit of claim 24 wherein, upon induction of light emission from said labels, a first of said different labels is an acridinium ester which emits light at a first wavelength and a second of said different labels is an acridinium ester having a greater degree of electronic conjugation than said first label, which emits light at a second wavelength longer than the first wavelength.

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